Arterial Dermatan Sulfate Proteoglycan Structure in Pigeons Susceptible to Atherosclerosis

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Arterial dermatan sulfate proteoglycans (DS-PG) were isolated from randomly bred White Carneau (RBWC) pigeons and a line of White Carneau pigeons (WC-2) genetically selected for increased atherosclerosis susceptibility. To characterize the basic proteoglycan (PG) structure and to identify structural differences that may relate to a specific arterial phenotype, embryos were labeled with radioactive sulfate in ovo and PG were isolated from aortas with 4.0 M GdnHCl. DS-PG were separated from chondroitin sulfate PG by gel chromatography and further purified by CsCl buoyant density ultracentrifugation and ion-exchange chromatography. The DS-PG monomers from the two genetic lines of pigeons eluted at different positions on two high pressure liquid chromatography (HPLC) gel permeation systems, which suggested a structural difference between the two monomers. Analysis of the monomer components showed a similar protein core molecular weight of 50,000 for each and a similar amino acid composition. Glycosaminoglycans (GAG) molecular weights were estimated to be 15,000 for WC-2 and 18,000 for RBWC. The findings suggest a basic difference in post-translational processing of PG in the two pigeon types which may reflect distinct functional properties associated with resistance or susceptibility to atherosclerosis. (Arteriosclerosis 5:101-109, January/February 1985)

Proteoglycans (PG), components of the extracellular matrix and cell surfaces, consist of sulfated linear polysaccharides, the glycosaminoglycans (GAG), covalently bound to a core protein. These carbohydrate macromolecules are vital in maintaining the structural integrity of the tissue. By their interactions with other connective tissue macromolecules such as collagen and elastin, PG form the ground substance that exists between cells. Distinct PG monomer types have been chemically isolated and described for artery, although the distribution of specific PG monomers in separate layers of the artery has not been fully established. Recently, Wagner et al. have shown that there are at least three major classes of proteoglycans in pigeon arterial tissue: a predominant large chondroitin sulfate-containing PG (CS-PG), a small dermatan sulfate-containing PG (DS-PG), and a polydisperse heparan sulfate PG.

The WC-2 pigeon, genetically selected from the randomly bred White Carneau pigeon (RBWC), has 2 to 3 times more severe and extensive atherosclerosis unexplained by any known risk factor but presumed to arise from differences in the artery wall. The artery phenotype has been linked to altered GAG amounts in subsequent studies that illustrate an increased content of dermatan sulfate in the progressing atherosclerotic plaque of the WC-2 pigeon. A possible mechanism of atherogenesis in the WC-2 pigeon is binding of plasma low density lipoprotein (LDL) to artery wall DS-PG. This hypothesis is based upon studies that have demonstrated in vitro the interaction of PG and LDL, and on studies demonstrating the isolation of PG-LDL complexes from atherosclerotic plaque. In view of the potential importance of the DS-PG in atherosclerosis, the present study was undertaken to characterize the basic structure of the intact PG monomers from WC-2 and RBWC arteries. We conducted the study on 18-day-old embryos labeled with radioactive sulfate in ovo and PG were isolated from aortas with 4.0 M GdnHCl.

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old embryos, to avoid confounding interpretation of the results, since squabs after hatch are fed "pigeon milk," which induces hypercholesterolemia. As well, older pigeons have naturally occurring atherosclerosis, which is more extensive in WC-2 pigeons.

Methods

Chemicals

Tryptamine hydrochloride, phenanthroline, Alcian blue, benzamidine-HCl, 6-amino-hexanoic acid, and 1-hexadecylpyridinium chloride (CPC) were obtained from Eastman Kodak Company, Rochester, New York. The CPC was recrystallized twice in acetone-water before use. Chondroitin ABC lyase (4.2.2.4) (Proteus vulgaris) and chondroitin AC lyase (4.2.2.5) (Arthrobacter aurescens) were purchased from Miles Laboratories, Elkhart, Indiana. Papain (2 × crystallized), D-glucuronic acid (Grade I) and bovine serum albumin (Fraction V) were obtained from Sigma Chemical Company, St. Louis, Missouri. Ultrapure GdnHCl, from Schwartz-Mann, Incorporated, Spring Valley, New York, was used without purification as the extraction solvent. Chondroitin 4-sulfate, chondroitin 6-sulfate, heparan sulfate, and hyaluronic acid were NIH standards obtained from M. B. Mathews and J. A. Cifonelli of the University of Chicago (Contract N01-AM-5-2205 from the National Institute of Arthritis, Metabolism, and Digestive Diseases). Chondroitin-6-sulfate from shark cartilage, chondroitin-4-sulfate from whale cartilage, dermatan sulfate from pigskin, and heparan sulfate from bovine kidney, all super-special grade from Miles Laboratories, Incorporated, were also used as standards for electrophoresis after verification that they migrated identically to the NIH GAG standards. 3H-sodium borohydride (341 mCi/mmole) and Aquasol-2 were purchased from New England Nuclear, Boston, Massachusetts, and sodium 125I-iodide (15.9 mCi/µg), from Amersham Corporation, Arlington Heights, Illinois. Flo-Scint II was obtained from Radiomatic Instruments, Tampa, Florida. All other chemicals were at least reagent grade and were obtained from Fisher. Pigeon eggs were obtained from the Research Farm of the Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, North Carolina.

Hexuronic acids were quantitated by the procedure of Blumenkrantz and Asboe-Hansen using D-glucuronic acid as the standard. Protein concentration was measured by the procedure of Lowry et al. using bovine serum albumin as the standard.

Preparation of Radiolabeled Embryos

Eggs were collected from specific mated pairs of WC-2 and RBWC pigeons and stored at 7° C for a maximum of 2 weeks to inhibit development. All eggs were turned daily and finally incubated in a sime forced-draft incubator at 38° C for 18 days. On the 17th day, the embryos of 10 eggs from each pigeon type were injected with 1.0 mCi of sterile sodium 35S-sulfate, sealed with wax, and placed back in the incubator. After 24 hours, arterial tissue that comprised the entire thoracic aorta and both brachiocephalic arteries was removed and cleaned of periadventitial tissue.

Extraction and Isolation of Proteoglycans

The tissue was minced and extracted for 24 hours at 4° C with 4.0 M GdnHCl in 0.05 M sodium acetate (pH 4.5) containing protease inhibitors as described previously by Salisbury and Wagner. The combined extracts for either WC-2 or RBWC were chromatographed on a column (0.9 × 55 cm) of Sepharose CL-4B (Pharmacia) eluted with 4.0 M GdnHCl in 0.05 M sodium acetate (pH 5.8). The column was eluted at a flow rate of 10 ml/hour, and 0.5 ml fractions were analyzed for radioactivity. The peaks of radioactivity were pooled and further purified by dissociative isopycnic CsCl centrifugation. The samples were loaded at a starting density of 1.40 g/ml. The gradient centrifugation was carried out at 100,000 × g (fmax) at 10° C, for 64 hours in a Beckman L8 preparative ultracentrifuge and Beckman type 65 rotor.

The bottom one-third of the gradient (density > 1.43 g/ml) was pooled, dialyzed versus 7.0 M urea, 0.05 M Tris-HCl (pH 7.5), and subjected to ion-exchange chromatography on DEAE-Sepharcel (Pharmacia). The column was eluted with three column volumes of 7.0 M urea, 0.05 M Tris-HCl (pH 7.5), followed by a step-wise gradient in the Tris-urea buffer with: 0.0 M NaCl, 0.15 M NaCl, 0.35 M NaCl, 0.50 M NaCl, and 1.0 M NaCl (Figure 1). The fractions were analyzed for radioactivity, dialyzed versus deionized H2O, and lyophilized. Both samples had a recovery of > 95% based on radioactive measurements before and after chromatography. The dried material was rehydrated in distilled H2O prior to analysis.

Isolation of Proteoglycan Glycosaminoglycans

GAGs were isolated by digestion of purified proteoglycan (100 µg hexuronic acid) with papain (0.17 units) in 0.08 M sodium acetate (pH 6.0), 5 mM cysteine-HCl, 1.5 mM EDTA at 25° C for 2 hours. The reaction mixture was dialyzed versus 0.01 M NaCl, 0.03 M Na2SO4 at 4° C overnight. Hexadecylpyridinium chloride was added to a concentration of 1%, the solution was incubated at 25° C for 24 hours and centrifuged at 1400 g for 30 minutes, and the pellet was dissolved in 2 M NaCl/absolute ethanol (100:15). Two volumes of absolute ethanol were added, and the sample was incubated at 25° C for 24 hours. The solution was centrifuged as above, and the GAG pellet was washed with 90%, 95%, and absolute ethanol and stored dried under vacuum until analyzed.
Identification of Glycosaminoglycans

The GAGs were identified following electrophoresis on cellulose acetate. GAG samples and standards (0.8 μg/μl) were electrophoresed in 0.1 M barium acetate (pH 8.6), at 8 mA per strip for 50 minutes. This system allowed for a complete separation of HS, DS, and CS. The cellulose acetate strips were cut longitudinally, and the standards were stained with Alcian blue (0.5% wt/vol), destained in methanol/water/acetic acid (10:9:1), and cleared in ethanol/acetic acid (3:1). Cellulose acetate strips containing samples were cut into pieces corresponding to the migration of standard GAG, dissolved in glacial acetic acid, and analyzed for 35S-radioactivity.

Preparation of Reductively 3H-Labeled Glycosaminoglycans

GAGs isolated from the PG were tritiated by the method of Tsuji et al. GAG (100 μg measured as hexuronic acid) was incubated with 4.0 μmol NaB[3H]4 (1.36 mCi) in 0.01 N NaOH for 4 hours at 25°C. Unlabeled NaBH4 (2 mg) was added, and the mixture was incubated for an additional 2 hours. The reaction mixture was passed through a 0.7 × 5 cm column of AG 50W-X 8 (H+ form) (BioRad) with H2O as the eluant. Any boric acid remaining was removed by repeated evaporation of added methanol.

Preparation of Radiolabeled Proteoglycans

Iodination was conducted by the chloramine-T method as described by Hunter and Greenwood. Briefly, the PG was dissolved in 0.25 M sodium phosphate (pH 7.5), and 32.0 μg of PG measured as hexuronic acid was radiiodinated following the addition of 0.5 mCi Na125I, 50 μg chloramine-T, 120 μg sodium metabisulfite, and 1.7 mg sodium iodide.

Preparation of Proteoglycan Core Proteins

Core proteins were prepared following treatment of samples with Chondroitin ABC (4.2.2.4) and AC (4.2.2.5) lyase digestion as described by Oike et al. except that the reactions were stopped by cold (5°C) instead of by the addition of GdnHCl.

Amino Acid Composition of Core Protein

A 5 to 10 μg sample of proteoglycan measured as protein was hydrolyzed in 6 N HCl and the solution was incubated at 100°C for 18 hours. The solution was evaporated to dryness and 0.1 N HCl was added. Dansylation of either amino acids following acid hydrolysis of PG or standard amino acid mixtures was carried out as described by Wiedmeier et al. Briefly, 1.5 mM of a standard amino acid mixture or 5 to 10 μg of PG hydrolysate was dissolved in 0.1 N HCl, NaHCO3 (pH 8.5), and dansyl chloride in acetone were added to final concentrations of 0.18 M and 8 mM, respectively. The pH was adjusted to 9.5 with 1.0 M NaOH, and the mixture was incubated at 25°C for more than 4 hours in the dark. The reaction mixture was centrifuged at 1600 g for 25 minutes, the pellet was discarded and 50 μl (equivalent to about 2 nmol of each amino acid) of the supernatant was used for analysis.

High Pressure Liquid Chromatographic Methods

Model 110A chromatographic pumps, a Model 153 analytical ultraviolet (UV) detector with a Model 420 gradient controller (Beckman Instruments, Incorporated) and a Model 3390 integrator (Hewlett-Packard, Corvallis, Oregon) were used for HPLC analysis.
Amino Acid Separation

Dansyl amino acids (Dns-aa) were separated on a 5 μm Ultrasphere-ODS C-18 column (250 × 4.6 mm ID, Beckman Instruments) at 25° C and detected by absorbance at 254 nm. In all runs, DNS-sarcosine was included as an internal standard. Two different elution gradients were used. The gradient system used to separate the more hydrophilic Dns-aa was: 10% to 20% solvent B in solvent A-1 over 5 minutes; 20% to 25% B over 45 minutes; 25% B for 10 minutes; then 25% B to 100% B over 1 minute.

The gradient system used to separate the more hydrophilic Dns-aa consisted of: 10% to 20% solvent B in solvent A-2 over 5 minutes; 20% B for 10 minutes; to 34% B over 45 minutes; to 55% B over 10 minutes; to 60% B over 15 minutes; to 100% B over 5 minutes; and 100% B for 10 minutes. Solvent A-1 was 20 mM sodium acetate (pH 3.3); solvent A-2 was 10 mM sodium acetate (pH 4.3)/tetrahydrofuran (THF)(95:5); solvent B was acetonitrile/THF (90:10).

Proteoglycan Monomer Hydrodynamic Size Determinations

Proteoglycan monomers were subjected to gel permeation chromatography by using two systems. One column was a TSK G4000 SW (300 × 7.5 mm ID, 13 μm Beckman) eluted with either 0.25 M Tris-phosphate (pH 7.6) or 4.0 M GdnHCl, 0.05 M sodium acetate (pH 5.8). The other column used was a TSK G5000 PW (600 × 7.5 mm I.D., 17 μm Beckman) eluted with 0.25 M Tris-phosphate (pH 7.6). Flow rates for both columns were 0.5 ml/minute. Fractions of 0.5 ml were collected from both columns and analyzed for radioactivity.

Proteoglycan Core Protein Molecular Weight Determinations

The molecular weight of the proteoglycan core proteins were estimated on OH-1000 and OH-500 columns (both 250 × 5 mm I.D., Brownlee) linked in series. Columns were calibrated with polystyrene standards (Supelco, Incorporated) and iodinated proteins of known molecular weights. The columns were eluted with 0.25 M Tris-phosphate (pH 7.6) at a flow rate of 0.5 ml/minute. Fractions of 0.5 ml were collected and radioactivity was measured by either a Gamma 4000 solid scintillation counter or an LS230 liquid scintillation counter (Beckman).

Glycosaminoglycan Molecular Weight Determinations

Tritiated GAG were chromatographed on an E-125 μ Bondagel column (Waters, Milford, Massachusetts). The mobile phase was HPLC grade H₂O at a flow rate of 0.5 ml/minute. The radioactivity was measured by using a Model HS-FLO-ONE flow through scintillation counter (Radiomatic Instruments). Tritiated GAG standards were used for calibration. In addition, ³H-labeled GAG were chromatographed on 1.5 × 90 cm columns of Sepharose 6B (Pharmacia) eluted with 0.2 M NaCl at a flow rate of 11.5 ml/hour. Fractions of 2.0 ml were collected and analyzed for radioactivity. Molecular weights were estimated by using $K_{av}$ values and standard published calibration curves.¹⁴

Results

The PG preparations of RBWC and WC-2 PG used in the study were calculated to be 78% and 82%, respectively, of the total artery pool of PG. These estimates were based upon measurements of nondialyzable $^{35}$S obtained following proteolysis of the artery after it was treated with GdnHCl. These...
percentages are similar to those reported in previous studies of pigeon artery PG.\textsuperscript{1-15} Proteoglycans isolated in Gdn-HCl and chromatographed under dissociative conditions on a CL-4B column and monitored by the \textsuperscript{35}S radioactivity are illustrated by Figure 2. Both the WC-2 and the RBWC samples had similar profiles illustrating three peaks of radioactivity. From other studies of pigeon artery PG, the first peak of radioactive material near the \(V_o\) has been identified as a CS-PG. The material with a \(K_m\) = 0.4 has been identified as DS-PG while the material at the \(V_i\) represents freely dialyzable (3500 MW membrane) radioactive sulfate. Small amounts of heparan sulfate PG have been described as localized between the first two peaks of radioactivity.\textsuperscript{1-15}

For each type of pigeon, material comprising the second peak of radioactivity of Sepharose CL-4B (47.4\% of the peak of RBWC and 47.7\% from WC-2) was pooled and subjected to isopycnic buoyant density ultracentrifugation in cesium chloride. Proteoglycan location, monitored by \textsuperscript{35}S radioactivity, is illustrated by Figure 3. The majority of the \textsuperscript{35}S radioactivity for both types of pigeons was in the bottom fraction, but it appeared that in the WC-2 pigeon there was a somewhat greater amount of PG isolated at a lower density (\(d = 1.42-1.36\)). The bottom one-third of the gradient was pooled, dialyzed and chromatographed on DEAE Sephacel. Figure 4 shows that in both preparations more than 95\% of the high molecular weight \textsuperscript{35}S-containing PG was eluted with 0.5 M NaCl.

After proteolysis of a sample of each PG and separation of individual GAG on cellulose acetate, it was determined that both preparations contained predominately DS (Table 1). It is unclear whether the small amount of CS detected is part of the DS-PG

![Figure 3.](image-url)

![Figure 4.](image-url)

![Table 1.](image-url)
monomers or is a contaminant resulting from overlap of PG types on Sepharose CL-4B. To further identify the $^{35}$S radioactivity located at the same position on cellulose acetate as authentic DS, we assessed the susceptibility of the radioactive material to chondroitinase ABC and AC. After these treatments, 85% of the PG from WC-2 and 90% of PG from RBWC was resistant to chondroitinase AC, but completely susceptible to chondroitinase ABC.

The elution profile of the PG from Sepharose CL 4B (Figure 2) suggested a possible size difference in the DS-PG monomers. The $K_v$ of the DS-PG peak from the WC-2 extract was 0.40, while the RBWC DS-PG eluted in a broader peak at a higher $K_v$ (0.45). Because of inadequate size resolution of large PG monomers on Sepharose CL-4B, the possible size difference was further examined following chromatography of radioiodinated $^{125}$I-DS PG on a TSK G4000 SW HPLC gel permeation column (Figure 5). The column profile indicated that the arterial DS-PG monomer from the WC-2 pigeon was larger than that from RBWC. The size difference was not explained by aggregate formation since the profiles for the monomers were the same under associative (0.25 M Tris-PO$_4$, pH 7.6) and dissociative (4.0 M GdnHCl) conditions. Distinct monomer sizes were confirmed by additional chromatography on a polymer-based TSK 5000 PW HPLC column with a higher size exclusion limit. In these runs the DS-PG from WC-2 and RBWC pigeons had a $K_v$ of 0.29 and 0.42, respectively.

To assess the possible structural basis of the size difference in PG monomers, the component parts of the macromolecule were examined. $^{125}$I-DS-PG core proteins were obtained following treatment with chondroitinase ABC and chromatographed on a calibrated gel permeation system consisting of an OH-1000 and an OH-500 column linked in series (Figure 6). The DS-PG core protein for both WC-2 and RBWC conditions.

![Figure 5. TSK G-4000 SW column profile of $^{125}$I-DS-PG monomer (0.5 M NaCl fraction of DEAE-Sephacel).](image)

![Figure 6. OH-1000 + OH-500 column profile of $^{125}$I-DS-PG protein core from RBWC (A) and WC-2 (B) pigeon arteries.](image)
RBWC pigeon arteries have a molecular weight of about 50,000. The amino acid compositions of the DS-PG core proteins were determined for further characterization of the molecules and were found to be similar (Table 2).

The glycosaminoglycan component of the monomers was examined to determine any structural differences between the two different lines of pigeons. The $M_r$ of the GAG chains isolated from the DS-PG monomers and reductively labeled with NaB[3H]$_4$, were estimated by chromatography on an E-125 $\mu$Bondagel gel filtration column (Figure 7) and on Sepharose 6B (data not shown). The apparent molecular weights of the GAGs from WC-2 and RBWC pigeon artery DS-PG were found to be 15,000 and 18,000, respectively, on both systems.

Discussion

The primary purpose of this study was to characterize the molecular structure of DS-PG from pigeon artery. This initial characterization was necessary before any subsequent functional studies concerning the DS-PG, and especially any specific role in atherosclerosis, could be conducted. In this study, it appears that arterial DS-PG monomers from RBWC and WC-2 pigeons differing in susceptibility to atherosclerosis are of different hydrodynamic sizes as evidenced by differing elution positions from HPLC gel filtration columns. Based upon the amino acid compositional data and the apparent molecular weight, the core proteins of the DS-PG monomer from both RBWC and WC-2 pigeon artery appear similar. Since there may be some small oligosaccharide chains attached to the core protein that remains after chondroitinase ABC treatment, the molecular weight of 50,000 may be a slight overestimate, although Chang et al. 16 have seen few or no oligosaccharide chains on a similar DS-PG isolated from monkey aortic smooth muscle cells in culture. The core protein of the DS-PG monomer was found to be smaller than the core protein of the CS-PG monomer (50,000 versus 160,000 for the pigeon artery CS-PG, data not shown). Since the amino acid composition is also different for the two proteins, this suggests there are two different genes regulating the synthesis of DS-PG and CS-PG monomers.

The size of the DS chains on the DS-PG monomer was determined by enzymatic digestion of the protein, isolating the carbohydrate moiety, and evaluating the GAG on two separate calibrated gel permeation systems. In both systems, these GAG
molecular weights were estimated to be 18,000 and 15,000 for the RBWC and WC-2 DS-PG, respectively. These molecular weights are smaller than the 39,000 reported for bovine aortic DS-PG, or for the cartilage DS-PG of cartilage. The exact reason for the distinct size difference of DS-PG in the two types of pigeon is not totally clear. The results of preliminary experiments on the proteoglycan to assess the extent of participation of serine and threonine of the core protein in glycosidic linkages suggest that there may be more GAG chains and/or non-GAG oligosaccharides as components of the WC-2 DS-PG monomer in contrast to the monomer from RBWC. So even though the GAG are shorter in the WC-2, there may be more per core of protein as compared to RBWC. More work remains to be conducted in this area before any firm conclusions can be drawn.

Another possible explanation of the difference in monomer hydrodynamic size is an alteration in the charge of the GAG chains. Reduced charge density, but unchanged GAG molecular weight, has been demonstrated in liver heparan sulfate of diabetic rats. If there were GAG charge differences in the two pigeon artery PG, this might have caused the DS-PG from the two lines to interact with the HPLC gel permeation columns in a disparate manner, resulting in an apparent size discrepancy. This appears to be unlikely because different elution buffers were used. In addition, any influences from altered GAG charge may be of a local nature since the overall charge of the monomer appears to be similar (based on elution from DEAE-Sephadex).

An early event in the pathogenesis of atherosclerosis in the pigeon is the deposition of plasma LDL lipid within the artery wall in the absence of any traditional risk factor. The WC-2 pigeon has been genetically selected for exacerbated atherosclerosis. As an example, in one study involving pigeons fed an atherogenic diet for 6 months, the cholesterol ester accumulation was 16.33 ± 2.87 and 5.03 ± 1.11 mg/g wet aorta in WC-2 and RBWC pigeons, respectively. The WC-2 pigeon is therefore a useful model for investigation of factors that render an animal susceptible to atherosclerosis, and that operate at the artery wall.

The lipid that deposits in the artery while atherosclerosis progresses accumulates both intracellularly and extracellularly in association with connective tissue components. The exact nature of the mechanism involved in the retention of LDL in the extracellular space has not been defined, but several investigators have suggested complexes involving PG and LDL. PG-LDL complexes have been demonstrated in vitro and complexed molecules consisting of PG and lipoprotein have been extracted from fatty streaks and fibrous atherosclerotic plaques.

The most consistent changes reported for the family of GAGs from artery PG have been increases in dermatan sulfate during the progression of atherosclerosis in species such as humans, monkeys, and pigeons. The study reported here implicates the DS-PG in atherogenesis by demonstrating that prior to the development of atherosclerosis, WC-2 embryos have structurally distinct PG monomers compared to RBWC. To our knowledge, this is the first report presenting data suggesting a qualitative difference in an artery PG monomer in an animal with a genetic predisposition for atherosclerosis. PG structure may be related to the increased atherosclerosis susceptibility in the WC-2 pigeon through an altered binding affinity to LDL. This possibility probably exists if the modification is in the carbohydrate moiety of the monomer, since the GAG is the major active component participating in the ionic association with LDL.

In comparison with properties of DS-PG reported from other tissues, the DS-PG from embryonic pigeon artery was found to have an apparent molecular weight larger than the M_r 130,000 for pig skin DS-PG and the M_r 190,000 for bovine aortic DS-PG (PG-B). The amino acid composition of PG-B is similar to that found for both pigeon arterial DS-PG cores (Table 2). The core protein molecular weights from CS-PG and DS-PG isolated from bovine sclera were reportedly 100,000 and 46,000, respectively. These measurements and the cartilage DS-PG core of 43,000 agree with the artery core protein molecular weights of 160,000 and 50,000 found in pigeons this study. These measurements are also consistent with the core protein and GAG chain molecular weight of 56,000 and 17,000, respectively, from calf-skin DS-PG. Since intact monomers from several different tissues vary in overall size, but appear to have a similar core protein molecular weight, variable DS-PG glycosylation is suggested.

There still remain many questions concerning the structure of arterial DS-PG. Whether the differences of DS-PG structure observed for the two lines of pigeons actually relates to an altered functional role of the PG in the development of atherosclerosis is of major interest. These functional studies, as well as an investigation of DS-PG structure in the atherosclerotic plaque, are vital for a complete understanding of the role of this connective tissue macromolecule in the pathogenesis of atherosclerosis.

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References


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