The Interaction between Human Plasma Lipoproteins and Connective Tissue Glycosaminoglycans*  

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PER-HENRIK IVERIUS

From the Institute of Medical Chemistry, University of Uppsala, S-751 22 Uppsala, Sweden

SUMMARY

The interaction between human plasma lipoproteins and glycosaminoglycans has been studied by use of gels consisting of cross-linked hyaluronic acid and of sulfated glycosaminoglycans covalently attached to agarose (Sepharose 4B). When chromatographed on a column of granulated hyaluronic acid gel at physiological pH and ionic strength, very low density (VLDL), low density (LDL), and high density (HDL) lipoproteins were shown to emerge with the void volume, indicating that no significant binding to the polysaccharide had occurred. Equilibration of the same lipoproteins with glycosaminoglycan-substituted agarose gels at pH 7.4 showed that VLDL as well as LDL were bound to gels containing heparin, dermatan sulfate, heparan sulfate, and chondroitin 4-sulfate, provided the ionic strength was sufficiently low. In contrast, HDL and acetylated specimens of VLDL and LDL did not bind to the gels at any ionic strength. The observed interactions have been interpreted as an ionic binding of positively charged amino groups on apolipoprotein B to negatively charged groups on the glycosaminoglycans.

The range of ionic strength at which each lipoprotein was released from a polysaccharide was relatively narrow and depended on the type of polysaccharide. For the release of half the amounts of VLDL or LDL, bound to gels of heparin, dermatan sulfate, heparan sulfate, and chondroitin 4-sulfate, ionic strengths of 0.26, 0.15, 0.09, and 0.08, respectively, were required. The results were interpreted in terms of an electrostatic binding between polyvalent anionic and cationic sites with the charge density of the polysaccharide being an important parameter for the strength of the ionic bond. At equal charge density, polysaccharides containing L-iduronic acid seemed to interact more strongly than those containing D-glucuronic acid.

Although the genesis of atherosclerosis is far from understood, certain causative factors as well as prominent features of the pathological process are known. Thus, the accumulation of lipids in the arterial intima seems to be a crucial step (1). The origin of the lipids is unclear, but it seems probable that they derive from plasma in which they are constituents of the lipoproteins. Histochemical evidence, obtained by the immunofluorescence technique, suggests that \( \beta \)-lipoprotein antigens are present in areas of the arterial intima, where extracellular lipid can be detected by fat stains (2, 3).

When it was discovered that \( \beta \)-lipoproteins could form complexes with sulfated polysaccharides (see e.g. Reference 4), interest was also directed towards the sulfated glycosaminoglycans and their possible role in the lipid accumulation. Most studies hitherto performed have been based on electrophoretic or turbidimetric methods (5-8). The biological significance of the cited work is questionable since physiological ionic conditions were not used. Furthermore, fractionated connective tissue glycosaminoglycans were studied only to a limited extent.

In the present study the interaction between glycosaminoglycans and human plasma lipoproteins has been investigated in more detail, and with particular regard to the effect of physiological pH and ionic strength. A novel method for interaction studies has been employed.

EXPERIMENTAL PROCEDURE

Materials—Fresh human blood, to which had been added 20% (v/v) of acid citrate dextrose (Acedex, Pharmacia AB, Uppsala, Sweden) as anticoagulant, was supplied by the courtesy of the Blood Center at the University Hospital in Uppsala. No selection was made with regard to blood groups.

Bovine serum albumin was purchased from Poviet Producten N. V., Amsterdam, Holland. Acetic anhydride was obtained from E. Merck AG, Darmstadt; agarose and antisera were obtained from Behringwerke AG, Marburg-Lahn in West Germany. Polyethylene glycol (PEG 20M) was supplied by Kebo AB, Stockholm, Sweden and a high molecular weight dextran fraction (FDR 1197, weight average molecular weight \( 12 \times 10^6 \)) by Pharmacia AB, Uppsala, Sweden.

Solid phase glycosaminoglycans were obtained in two different ways. The sulfated glycosaminoglycans, which have a serine or a peptide residue in their link region and thus a free amino group (9), were covalently attached to agarose gels (Sepharose 4B) activated by cyanogen bromide (10). The batches used in the present work have been described previously (11). They contained pig intestinal mucosal heparin (Hep-II-Seph. 4B-VI), human aortic heparan sulfate (HS II Seph. 4B-I), bovine aortic dermatan sulfate (DS-III-Seph. 4B-II), and bovine...
by a modification of the method of Noble (23), using the LKB University lipoproteins (d 1.125 to 1.20 g per cm³). CEC, critical electrolyte concentration of the gel grains was 3.6% (w/v) when measured at different color yield of apolipoprotein was made according to strable link region (9), was provided as a cross-linked, granulated gel by Dr. K. Hellsing of this Institute. The polymer presented in Table I. Hyaluronic acid, which has no demonstrated link region (9), was provided as a cross-linked, granulated gel by Dr. K. Hellsing of this Institute. The polymer concentration of the gel grains was 3.6% (w/v) when measured at pH 8.5 and ionic strength 0.1 (14).

Analytical Methods—Protein was determined by the method of Lowry et al. (15) as modified by Gustafson et al. (10). Since bovine serum albumin was used as a standard, correction for a different color yield of apolipoprotein was made according to Margolis and Langdon (17). The correction factor has been determined for the apolipoprotein of LDL but should be valid also for other apolipoproteins. The ninhydrin reaction was used to estimate free amino groups (18). Turbid samples were clarified by filtration through Celite prior to the photometric reading.

Lipids were extracted from suitable sample volumes (0.6 ml or less) with chloroform-methanol, 2:1 (v/v) in a final volume of 25 ml. After filtration, aliquots were subjected to analyses. Triglyceride was determined against a standard of tripalmitin (19); the results are expressed as triolein. The method of Abell et al. (20) was used for assays of total cholesterol, while that of Sperry and Webb (21) was adopted for free cholesterol. Cholesterol ester was calculated by difference and expressed as cholesteryl oleate. Analysis for lipid phosphorus was performed by the Bartlett method (22). The factor 25 was used for conversion of lipid phosphorus to phospholipid.

Electrophoresis of lipoproteins was carried out on glass slides by a modification of the method of Noble (23), using the LKB Immunophor Apparatus (LKB-Produkter AB, Stockholm, Sweden). The supporting gel consisted of 0.6% agarose and 1% albumin in 0.05 M veronal buffer, pH 8.6. The separation was carried out at a potential gradient of 5 volts per cm for 2 hours at room temperature. After fixation and drying, the slides were stained for 16 hours at room temperature in 60% (v/v) of ethanol saturated with fat red 7B and oil red O. Immunodiffusion analysis (24) was performed with gels of 1% agarose in 0.05 M veronal buffer, pH 8.6. Rabbit antiserum against human serum albumin, α1-lipoprotein, and β-lipoprotein were employed. Samples to be analyzed were applied at four dilutions (1/1 to 1/729).

Preparation of Lipoprotein Fractions—All ultracentrifugations were carried out in Beckman Spinco preparative ultracentrifuges, models L or L2-65B. If not stated otherwise, the temperature setting was 17° and the velocity was 50,000 rpm. The densities of solutions to be centrifuged were adjusted either with solid KBr or with a NaCl-KBr-containing solution (25), and were then checked by pycnometry at 20°. Thermostat (0.1 mg per ml) and EDTA (0.1 mg per ml) were added to all solutions prior to ultracentrifugation (26). After centrifugation the contents of the tubes were divided into a top and a bottom fraction at the clear middle zone.

Plasma was obtained from blood by centrifugation at 3500 x g for 15 min at 4°. After further separation at d 1.20 for 24 hours in rotor 50.1, the top fraction containing the lipoproteins was concentrated to 95 ml in order to fit the rotor 65, which was used in all of the following centrifugation steps. After adjusting the density to 1.06 by dialysis, VLDL was isolated in the top fraction by centrifugation for 12 hours. Removal of the fraction of Sf > 400 was carried out as described by Gustafson et al. (16) by using rotor 65 at 17,500 rpm. Finally, a recentrifugation at d 1.006 followed.

The LDL was isolated at d 1.063 and recentrifuged consecutively at d 1.019 and 1.063 for 12 hours. The fraction of density lower than d 1.019 was discarded.

### Table I

<table>
<thead>
<tr>
<th>Glycosaminoglycan concentration in wet gel</th>
<th>Molecular weight</th>
<th>Weight</th>
<th>Chain</th>
<th>Disaccharide residue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Weight average</td>
<td>Number average</td>
<td>mg/ml</td>
<td>%</td>
</tr>
<tr>
<td>Heparin (Hep-II)</td>
<td>12,800</td>
<td>10,500</td>
<td>0.42</td>
<td>3.3</td>
</tr>
<tr>
<td>Heparan sulfate (HS-II)</td>
<td>58,300</td>
<td>49,500</td>
<td>0.27</td>
<td>4.7</td>
</tr>
<tr>
<td>Chondroitin sulfate (CS-I)</td>
<td>37,100</td>
<td>27,900</td>
<td>0.28</td>
<td>7.5</td>
</tr>
<tr>
<td>Dermatan sulfate (DS-III)</td>
<td>41,500</td>
<td>39,600</td>
<td>0.25</td>
<td>6.1</td>
</tr>
</tbody>
</table>

a Per cent of dry weight.
b Molar ratio (11).

### Notes

1. The abbreviations used are: LDL, low density lipoproteins (d 1.019 to 1.063 g cm⁻³; Sf = 0 to 12); VLDL, very low density lipoproteins (d < 1.006 g cm⁻³; Sf = 20 to 400); HDL, high density lipoproteins (d 1.063 to 1.125 g cm⁻³); HDL₉, high density lipoproteins (d 1.125 to 1.200 g cm⁻³). CEC, critical electrolyte concentration.

2. A. Gustafson, personal communication.
TABLE II

Chemical composition of lipoprotein fractions

Values are expressed as percentage of the sum of all constituents analyzed.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Protein*</th>
<th>Phospholipid</th>
<th>Triglyceride</th>
<th>Cholesterol*</th>
<th>Free cholesterol</th>
<th>Total cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL</td>
<td>7%</td>
<td>20%</td>
<td>51%</td>
<td>16%</td>
<td>6%</td>
<td>15%</td>
</tr>
<tr>
<td>Acetylated VLDL</td>
<td>5%</td>
<td>19%</td>
<td>69%</td>
<td>9%</td>
<td>6%</td>
<td>12%</td>
</tr>
<tr>
<td>LDL</td>
<td>15%</td>
<td>24%</td>
<td>9%</td>
<td>43%</td>
<td>8%</td>
<td>33%</td>
</tr>
<tr>
<td>Acetylated LDL</td>
<td>15%</td>
<td>28%</td>
<td>12%</td>
<td>37%</td>
<td>8%</td>
<td>30%</td>
</tr>
<tr>
<td>HDL</td>
<td>42%</td>
<td>30%</td>
<td>8%</td>
<td>18%</td>
<td>2%</td>
<td>12%</td>
</tr>
</tbody>
</table>

* Determined by the Lowry method against a standard of bovine serum albumin and corrected for the lower color yield of this standard as compared to that of the lipoprotein (17).

b Calculated as lipid phosphorus × 25.

c Calculated as triolein.

d Calculated as cholesteryl oleate.

The HDL remained in solution after removal of HDL by centrifugation at d 1.125 for 24 hours. The former fraction was finally purified by another centrifugation for 24 hours at d 1.20.

The lipoprotein fractions were stored at 4°C after dialysis against 0.15 m NaCl containing EDTA (0.1 mg per ml). Thiourea was also added (0.1 mg per ml) after the chemical analysis of the fractions had been performed.

Acetylation of LDL and VLDL—The acetylation procedure of Fraenkel-Conrat (18) was adopted. LDL (190 mg in 2.5 ml) or VLDL (138 mg in 3 ml) were mixed with equal volumes of saturated sodium acetate and chilled in an ice bath. Acetic anhydride (50 μl) was added four times at 15-min intervals. Fifteen minutes after the last addition, the lipoproteins were dialyzed against the appropriate buffer for the experiments.

Gel Chromatography—A granulated gel of cross-linked hyaluronic acid was packed into a column (1.1 x 18.5 cm) and equilibrated with 3 mM veronal-1 mM EDTA-HCl buffer (pH 7.4) containing 0.151 m NaCl (ionic strength 0.160). The void volume (V0) and the total volume of the column were determined after the elution pattern of dextran and tritiated water, respectively (27, 28). VLDL (4.6 mg), LDL (7.6 mg), or HDL (7.4 mg) were applied in a volume of 0.1 ml and were then eluted at a flow rate of 5.2 ml per hour. Effluent fractions of 0.6 to 0.7 ml were collected and analyzed for protein by measuring the absorbance at 280 nm after dilution with buffer (1:1).

Equilibration of Lipoproteins with Glycosaminoglycan-substituted Agarose Gels—Agarose gel, suspended in distilled water, was distributed in a series of small test tubes. After centrifugation (135 x g for 15 min), the supernatants were carefully removed and the gel contents of the tubes were adjusted to 0.50 g. The gels were then equilibrated with buffers of ionic strength varying between 0.01 and 1.00 by washing four times with 2 ml of 3 mM veronal-HCl (pH 7.4) containing varying amounts of NaCl. In addition, the buffers contained either 1 mM EDTA or 2.5 mM CaCl2-1 mM MgCl2. After the last washing, the supernatants were carefully removed and all of the tubes were charged with 0.25 ml of lipoprotein solution dialyzed against buffers containing either EDTA (ionic strength 0.01) or divalent cations (ionic strength 0.02). Finally, 0.25 ml of buffer containing varying amounts of NaCl was added to each tube in order to obtain the desired ionic strength of the equilibrium mixture. The contents were carefully mixed four times within 1 hour, left at 4°C overnight, then mixed again and finally centrifuged before the supernatants were subjected to assays for total cholesterol.

RESULTS

Properties of Native and Acetylated Lipoprotein Fractions—The chemical compositions of the lipoprotein fractions are given in Table II.

The extent of acetylation of LDL and VLDL was estimated by...
performing the ninhydrin reaction on acetylated as well as on nonacetylated samples. The color yield in the ninhydrin reaction of each sample was compared to the corresponding lipoprotein concentrations as calculated from cholesterol determinations. The acetylated specimens of LDL and VLDL yielded only 10.3 and 4.4%, respectively, of the color produced by equivalent amounts of nonacetylated lipoproteins.

Electrophoresis (Fig. 1) of HDLb, VLDL, and LDL indicated pure fractions with α1-, pre-β-, and β-mobilities, respectively. The acetylated specimens of VLDL and LDL moved considerably faster than their nonacetylated counterparts, indicating that positively charged groups had been blocked.

Immunodiffusion experiments showed that the LDL and VLDL fractions reacted strongly with anti-β-lipoprotein and very faintly with anti-α1-lipoprotein. The HDLb fraction gave a strong reaction with anti-α1-lipoprotein and a very faint reaction with anti-β-lipoprotein. Albumin was not detected in any of the fractions.

Chromatography of Lipoproteins on Hyaluronate Gel—The chromatograms in Fig. 2 indicate that VLDL, LDL, and HDLb emerged with the void volume. It was, therefore, concluded that no significant binding of the lipoproteins to the hyaluronate gel occurred under the conditions of physiological pH and ionic strength.

Ionic Interactions between Lipoproteins and Sulfated Glycosaminoglycans—After the equilibration of a lipoprotein species with a polysaccharide- or ethanolamine-substituted gel at varying ionic strength, the lipoprotein concentration in the supernatant was estimated by total cholesterol assay. The supernatant concentration was then plotted against the ionic strength. The lipoprotein concentration recorded in the supernatant at the highest ionic strength (1.00) was set to 100%. Due to exclusion of soluble lipoprotein from the gel phase, it was generally in the order of 1.2 times higher than the total concentration of lipoprotein in the equilibration mixture.

The experiments performed with VLDL and LDL in the presence of EDTA, shown in Figs. 3 and 4, respectively, can be discussed together since both lipoprotein classes behaved almost identically. Three different ionic strength-intervals could be distinguished with regard to the effect on the lipoprotein concentration in the liquid phase. In a broad interval at high ionic strength, fairly constant lipoprotein levels were recorded. Here, presumably no lipoprotein was bound. Within a narrow ionic strength interval, which was characteristic of each polysaccharide, the extent of binding changed from negligible to maximal. Finally, at the very lowest ionic strength the lipoprotein concentrations in the liquid phase increased again. This decrease in binding capacity may tentatively be ascribed to an increased exclusion of macromolecules from the gel, due to sterics as well as ionic factors. The former should be important since charged polysaccharide chains attain an expanded configuration at low ionic strength (29). The latter may cause an increased repulsion between negative charges on the polysaccharide and the lipoprotein, at least in the case of LDL, which should bear an overall negative charge at pH 7.4 since its isoelectric point is lower (4). The three intervals of ionic strength,
FIG. 4. Binding of LDL to ethanolamine- or glycosaminoglycan-substituted gels. The symbols and conditions are identical with those described in the legend to Fig. 3. All tubes contained 3.0 mg of lipoprotein, except those containing ethanolamine and heparan sulfate, to which 3.4 mg of lipoprotein were added.

mentioned above, were also traced in the experiments with the ethanolamine-substituted gel. However, only a minor reduction of lipoproteins in the liquid phase was observed when the ionic strength was lowered. It is therefore concluded that at appropriate conditions of ionic strength the sulfated glycosaminoglycans, bound to the gel matrix, possessed a strong capacity to bind VLDL and LDL. The binding capacity, although small, of the ethanolamine-substituted gel might be ascribed to the presence of small amounts of sulfate in the agarose (11).

The ionic strength dependence of the binding of VLDL and LDL in the intermediate interval has a striking similarity to the reaction between glycosaminoglycans and cationic detergents (30). The salt concentration in the middle of such a narrow interval, in the case of detergents, has been denoted a critical electrolyte concentration. In the experiments depicted in Figs. 3 and 4, the CEC's were intrapolated from the curves at the levels halfway between the highest and the lowest concentrations of the curves. VLDL as well as LDL showed CEC's of 0.26, 0.15, 0.09, and 0.08 with heparin, dermatan sulfate, heparan sulfate, and chondroitin sulfate, respectively. Ideally, when the supernatant concentration varies between 0 and 100%, the CEC is the ionic strength at which the supernatant concentration attains 50%. However, since nonideal conditions prevailed, the adopted method of evaluation seems satisfactory.

Equilibration of LDL with the various gels in buffers containing 2.5 mM CaCl₂-1 mM MgCl₂ instead of 1 mM EDTA resulted in CEC's identical with those obtained in the presence of EDTA.

When HDL₃ was equilibrated with gels at various ionic strengths (Fig. 5A), the curves obtained with the four polysaccharide-substituted gels were similar to the curve obtained with the ethanolamine-substituted gel. Thus, there was no significant binding of HDL₃ to the sulfated glycosaminoglycans at any ionic strength.

The acetylated specimens of VLDL and LDL were only equilibrated with the dermatan sulfate- and heparin-substituted gels (Fig. 5, B and C). Since the curves were similar to those obtained with nonacetylated lipoproteins and ethanolamine-substituted gel (Figs. 3 and 4), it is concluded that the acetylation procedure blocked the binding of VLDL and LDL to the sulfated glycosaminoglycans.

FIG. 5. Equilibration of A, HDL₃ (3.7 mg); B, acetylated VLDL (3.4 mg); C, acetylated LDL (0.9 mg); with various gels under conditions identical with those described in legend to Fig. 3. ○—○, ethanolamine; □—□, chondroitin sulfate; △—△, heparan sulfate; ●—●, dermatan sulfate; ■—■, heparin.

DISCUSSION

Nature of Cationic Sites on Lipoproteins—The interaction demonstrated between the sulfated glycosaminoglycans and VLDL or LDL was strongly influenced by salt concentration and should, therefore, be ionic (30). Treatment of the lipoprotein with acetic anhydride, which is known to block free amino groups (18, 31), inhibited the interactions completely. Hence, the ionic bonds are probably established between the negatively charged groups of the polysaccharide and positively charged amino groups of the lipoprotein.

Free amino groups in lipoproteins are provided by lysine residues, amino-terminals of polypeptide chains, phosphatidylserine, and phosphatidylethanolamine. The last two substances account for less than 5% of all positive charges of the phospholipids in LDL (17). The involvement of phospholipids in the interactions observed with VLDL and LDL seems improbable since HDL₃ did not bind to the glycosaminoglycans despite its higher phospholipid contents. The alternative interacting components are the apolipoproteins. LDL contains almost exclusively apolipoprotein B, while VLDL in addition has two others, named A and C. The latter two are also constituents of HDL₃ (32), which did not bind to the polysaccharides. Therefore, it seems likely that the binding of VLDL as well as LDL to the glycosaminoglycans is mediated by apolipoprotein B. The finding of identical CEC's for LDL and VLDL with each particular polysaccharide lends further support to this statement.

The present work, performed by a new technique, corroborates conclusions recently drawn by others. Levy and Day (33), as well as Nishida and Cogan (34), studied the interaction between...
Although the experiments with hyaluronic acid were performed at a physiological ionic strength only, and with a technique different from the other experiments, it seems reasonable to add this polysaccharide at the end of the sequence. The above order differs from that recently suggested by Srinivasan et al. (35). Since that work was carried out by a different technique, using incompletely characterized polysaccharides, a detailed comparison with the present work is precluded.

If the experimental values obtained at the lowest ionic strengths are disregarded (see under "Results"), the interaction curves obtained with LDL and VLDL have approximately a sigmoid shape, indicating that the binding and the dissociation of the lipoproteins is a cooperative phenomenon (36). The curves may therefore be described by the Hill equation (36)

$$\log \frac{\alpha}{1-\alpha} = z \log [M+Q^-] - \log K$$

where $\alpha$ is the fraction of unbound lipoprotein, $z$ is the Hill coefficient, $[M+Q^-]$ is the concentration of monovalent salt and $K$ is a constant. Assuming that all lipoprotein is free ($\alpha = 1$) at an ionic strength of 1.0, the Hill coefficient ($z$) was calculated for the various experiments with LDL (Fig. 6). The consecutive order of the polysaccharides with regard to their $z$ values turned out to correspond to the order with regard to their CEC's, which implies that also the $z$ values can be used as a measure of the affinity between the lipoprotein and the polysaccharide. Ideally, the various $z$ values may reflect the number of ion pairs cooperating to form a polyvalent binding site between the polysaccharide and the lipoprotein. Alternatively, the $z$ values may represent the number of mono- or polyvalent cooperating binding sites. Although the nature of the cooperative mechanism cannot be elucidated by the present experiments and, in addition, the $z$ values certainly are subject to experimental error, the present findings, nevertheless, may give clues to how different affinities for a certain lipoprotein may be achieved.

With two exceptions, discussed below, the affinity between a polysaccharide and a lipoprotein seems to be dependent on the charge density of the polysaccharide. The latter parameter may exert its influence in different ways. First, the charge density may affect the binding properties of each particular negative group. Second, a polymer of high charge density should offer a greater number of binding positions along the chain than one of low charge density and thus influence the binding by the law of mass action. Third, the charge density may influence the degree of cooperativity in the binding. If the binding site on the lipoprotein consists of a small cluster of positive charges, a polysaccharide of high charge density should cover more of these charges than one of low, provided that the charge density on the lipoprotein equals or exceeds that of the highly charged polysaccharide. This last alternative is presumably the most important one.

There are two exceptions to the importance of the charge density in the establishment of different affinities for a lipoprotein. First, the dermatan sulfate shows significantly higher $z$ values than the chondroitin sulfate, although both

$z$ It seems reasonable to relate the CEC's to a parameter which is proportional to the total length of all glycosaminoglycan chains in a gel rather than to the number of separate chains.

Fig. 6. Calculation of the Hill coefficients for the binding of LDL to heparin (Hep), dermatan sulfate (DS), heparan sulfate (HS), and chondroitin sulfate (CS). The straight lines were calculated on a desk-top computer according to a least squares program. The slopes ($z$), which equal the Hill coefficients, and the correlation coefficients ($r$) are indicated in the figure.
polysaccharides contain equal amounts of sulfate (Table I). Second, the CEC and z values of the heparan sulfate are of the same order as those of chondroitin sulfate, despite a lower degree of sulfate substitution (Table I). Since dermatan sulfate and chondroitin sulfate differ only with respect to the uronic acid composition (9), it seems valid to assume that the presence of d-iduronic acid in dermatan sulfate accounts for the higher reactivity of this polysaccharide. The possibility that also heparan sulfate contains L-iduronic acid (9) might explain the anomalous behavior of this polysaccharide. If a cooperation of ion pairs accounts for the establishment of different affinities, the steric arrangement of the charges on a polysaccharide chain is certainly important. Thus, the local charge density in a certain direction from the polysaccharide chain may be higher in polysaccharides containing d-iduronic acid than in those containing d-glucuronic acid, at equal chemical charge densities.

Aspects on Atherosclerosis—As shown above, two lipoproteins (VLDL and LDL), known to be atherogenic (37), bind at physiological pH and ionic strength to one of the glycosaminoglycans (dermatan sulfate) found in the arterial wall. This observation demands some remarks concerning arterial polysaccharides.

The extracellular glycosaminoglycans hitherto identified in human aorta are hyaluronic acid, dermatan sulfate, heparan sulfate, chondroitin 6-sulfate (38), and chondroitin 4-sulfate (39). There is, however, no convincing evidence for the presence of heparin and keratan sulfate. Unfortunately, chondroitin 6-sulfate has not been included in this work. Since it is similar to chondroitin 4-sulfate in many respects (40), it is not expected to behave much differently with regard to its interaction with lipoproteins. Data in the literature, concerning the polysaccharide composition of the arterial wall in various states of age and health, are conflicting. The discrepancies may be due to differences of methodology as well as sample selection. However, the maturation of an individual means that the polysaccharides digestible with testicular hyaluronidase, i.e. hyaluronic acid and chondroitin sulfate, are more or less replenished by the hyaluronidase-resistant ones, namely heparan sulfate and dermatan sulfate (38). An interesting finding in this context was made by Kumar et al. (41), who analyzed the glycosaminoglycan composition in human arterial intima and externa in different stages of atherosclerosis. The dermatan sulfate was almost exclusively confined to the fatty streaks and pre-stages of this lesion. If lipoprotein deposition in the arterial wall is related to the polysaccharide composition, there may be two alternative explanations to the phenomenon cited. Dermatan sulfate may appear in the fatty streaks as part of a tissue reaction, due to the presence of lipid. According to the other alternative, which is favored by the present work, lipoproteins accumulate and form fatty streaks where dermatan sulfate is abundant.

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