Molecular Modeling of Protein-Glycosaminoglycan Interactions

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Forty-nine regions in 21 proteins were identified as potential heparin-binding sites based on the sequence organizations of their basic and nonbasic residues. Twelve known heparin-binding sequences in vitronectin, apolipoproteins E and B-100, and platelet factor 4 were used to formulate two search strings for identifying potential heparin-binding regions in other proteins. Consensus sequences for glycosaminoglycan recognition were determined as \([X-B-B-X-B-X] \) and \([X-B-B-B-X-X-B-X] \) where \(B \) is the probability of a basic residue and \(X \) is a hydrophatic residue. Predictions were then made as to the heparin-binding domains in endothelial cell growth factor, purpurin, and antithrombin-III. Many of the natural sequences conforming to these consensus motifs show prominent amphipathic periodicities having both \( \alpha \)-helical and \( \beta \)-strand conformations as determined by predictive algorithms and circular dichroism studies. The heparin-binding domain of vitronectin was modeled and formed a hydrophilic pocket that wrapped around and folded over a heparin octasaccharide, yielding a complementary structure. We suggest that these consensus sequence elements form potential nucleation sites for the recognition of polyanions in proteins and may provide a useful guide in identifying heparin-binding regions in other proteins. The possible relevance of protein-glycosaminoglycan interactions in atherosclerosis is discussed.

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There is considerable interest in elucidating the specific mechanism(s) by which heparin and other glycosaminoglycans (GAG) interact with proteins to regulate metabolic processes in both normal and disease states. Examples of these processes include hemostasis,\(^1\) cell-substrata adhesion,\(^2\) smooth muscle cell proliferation,\(^3\) lipolytic enzyme activity,\(^4\) lipoprotein-arterial wall interactions,\(^5,6\) and regulation of growth factors in angiogenesis.\(^7,8\) However, the structural heterogeneity of the acidic mucopolysaccharides with respect to size, carbohydrate composition and charge,\(^9\) and the variety of proteins that are known to bind to these molecules have complicated a detailed molecular analysis of protein-GAG interactions. The most studied system, binding of heparin to a specific domain or site on human antithrombin-III (AT-III), involves a unique carbohydrate structure representing only a fraction of the total heparin.\(^10\) The exact amino acid residues on AT-III or other proteins which comprise their heparin-binding domains have not been fully elucidated.

To understand the molecular recognition of heparin by proteins, we recently determined the structure of the heparin-binding regions of apolipoprotein (apo) B-100, the major protein constituent of human plasma low density lipoproteins (LDL). The structure of these regions was of particular interest as we hypothesized that they could mediate the interaction of LDL with acidic mucopolysaccharides of arterial tissue. We showed that LDL contains five to seven heparin-binding sites on the lipoprotein surface.\(^11\) The heparin-binding cyanogen bromide peptides of apo B-100 were isolated, and their amino acid sequences were determined.\(^12\) These peptides account for five domains of high positive charge density that we suggest mediate the lipoprotein's interaction with glycosaminoglycans. These same regions were also identified by Weisgraber and Rall.\(^13\) We noticed\(^14\) that these domains in apo B-100 have a sequence similarity to the heparin-binding domains of apo E\(^15\) and human vitronectin (Vn)\(^16\) with respect to the organization of basic and hydrophatic residues. The present study extends our initial observations to other proteins whose functions are modified by heparin. We show that all these proteins contain unique consensus sequences of amino acid residues that are potentially involved in heparin binding.

**Methods**

Secondary structure predictions of Vn peptide conformations were performed by the MSEQ program\(^17\) based on Chou-Fasman algorithms\(^18\) and by the method of Garnier et al.\(^19\) Both methods gave comparable predictions of predominant \( \beta \)-strand and \( \beta \)-turn structures, and circular dichroism studies on consensus peptides in the presence of heparin were consistent with these predictions. From these data, a starting structure was devised for the Vn peptide in the three-dimensional modeling studies on heparin binding. Heparin was modeled based on the consid-
orations of Cowan et al.24 The heparin disaccharide repeat of 1,4-linked 2-sulfate-α-L-idopyanosyluronic acid and 2-deoxy-2-sulfamino-α-D-glucose-6-sulfate was constructed from cyclohexane by the addition of the appropriate hydrophilic side chains and replacement of one of the tetracyclic methylene groups with an oxygen.

Molecular modeling of Vn-heparin interactions was performed with an Evans and Sutherland PS-350 Graphics System. Molecular structure data on both Vn and heparin were analyzed using the Insight and Discover programs25-28 from Biosym Technologies, Incorporated. Individual structures were built with Insight software and geometrically optimized with the force field in Discover.29-33 The potential energies of the Vn peptide and heparin structures were separately minimized. Heparin and peptide structures were then visually docked by using Insight on an Evans and Sutherland graphics workstation. The resultant complex was geometry-optimized using the Discover force field and was subjected to molecular dynamics simulation on a Vax and a Cray supercomputer. Molecular dynamics were performed as follows: the complex was "heated" to 600° K and was allowed to equilibrate for 500 fs. The atomic trajectories were than sampled every 100 fs thereafter for 5 to 7.5 ps. The resulting structure (geometry) obtained at each 100 fs sampling point was cooled to 300° K and was allowed to equilibrate over an additional 100 fs of molecular dynamics simulation. The resultant complex was then geometry-optimized to yield a final structure.

Amphipathic periodicities of peptides were analyzed by the methods of Eisenberg et al.34 and Schiffer and Edmunson.35

Results
Consensus Sequence Analyses of Heparin-binding Regions

The heparin-binding domains of human apo B-100,18,17 apo E,19,16 Vn,29 and platelet factor 4 (PF-4)24 consist of extended sequences of basic residues. The three-dimensional molecular interaction of PF-4 with heparin has been modeled based on the X-ray crystallographic structure of the tetrameric form.24 A similar model for the binding of heparin to β-thromboglobulin based on the structural homology of this protein with PF-4 was also proposed.24 The heparin-binding regions of these proteins are characterized by clusters of arginines and lysines. These clusters form centers of high positive charge density that electrostatically interact with the acidic groups of mucopolysaccharides, such as heparin. A feature of these natural sequences is the interspersion of basic and hydrophilic residues. We have investigated whether the similarity in organization of basic and hydrophilic residues in the heparin-binding domains of these proteins could be used to identify the basic regions in other heparin-binding proteins that function in polyanion recognition.

As indicated above, sequence similarities among the heparin-binding domains of Vn, apo E, apo B-100, and PF-4 with respect to the organization of basic and hydrophilic residues were noted. These sequences are shown in Tables 1 and 2. The basic regions of other known heparin-binding proteins are also presented; however, these regions have not been shown experimentally to bind heparin. The selection of these sequences was based on their similarity to the experimentally defined heparin-binding domains in Vn, apo E, apo B-100, and PF-4, and, thus, represent putative heparin-binding regions. The regions shown are characterized by two consensus sequences [−X-B-B-X-B-X−] (Table 1) and [−X-B-B-X-B-X−] (Table 2), where B is the probability of occurrence of a basic residue and X is a hydrophilic residue. The consensus sequences were determined by the matrix analyses shown in Tables 3 and 4. The matrices show the frequency of occurrence of any given residue at each position within the [−X-B-B-X-B-X−], i.e., −3 to +3 positions, and [−X-B-B-X-B-X−], i.e., −4 to +4 positions. With respect to the [−X-B-B-X-B-X−] consensus sequence (see Table 3), Lys is the most common residue at positions −2 and −1, whereas Arg predominates at +2. His is infrequently used at these positions. The −2, −1, and +2 positions show exclusive usage by basic residues, whereas the region of the consensus has virtually no acidic residues. The [−X-B-B-X-B-X−] consensus sequence (see Table 4) shows Lys as the predominant basic residue at the −2, −1, and +3 [−B−] positions. Arg is the second most utilized, with His having infrequent usage. The sequences within the consensus are relatively abundant in Asn, Ser, Ala, Gly, Ile, Leu, and Tyr and exhibit a very low occurrence of Cys, Glu, Asp, Met, Phe, and Trp. The −2 and +3 [−B−] positions contain only basic residues, whereas the −3 and −1 [−B−] positions have the greatest variation, with 16 and 17 out of 28 residues being basic, i.e., −60% usage at positions −3 and −1 versus 100% at positions −2 and +3. Although the −3 and −1 positions show a lower frequency of basic residues, either one or both of these positions are used. Thus, the consensus represents either a stretch of di- or tribasic residues separated by two or three hydrophilic residues terminated by one or more basic residues. The alignments shown maximize the sequence similarities among the basic regions of the various proteins with the known heparin-binding regions of the reference proteins.

Studies with synthetic peptides corresponding to specific consensus sequences show that these structures can function in polyanion recognition.35-37 For example, a synthetic peptide containing the consensus [−X-B-B-X-B-X−] that includes residues 3364 to 3371 of apo B-100 (T-R-K-R-G-I-L-K-L) binds heparin at the same ionic strength conditions as LDL37 and corresponds to a segment of a heparin-binding CNBr fragment isolated from apo B-100.16 Similarly, a synthetic peptide of apo E, comprising the LDL receptor binding domain38 and having essentially the same sequence within the consensus region as the apo B-100 synthetic peptide (see Table 2), accounts for the major heparin-binding activity of apo E.18,19 Using synthetic peptides with successive deletions from the amino terminus, we showed that residues 144 to 147 [L−R−K−R] of apo E were essential for heparin-binding activity.18 These residues represent the [−X-B-B-B−] elements of the consensus sequence [−X-B-B-B-X-B−X−] shown in Table 2 and likely account for a significant amount of the heparin-binding activity in a major thromboytic fragment from apo E.18 Monospecific antibodies to
Table 1. Sequence Similarities of Heparin-binding Proteins

| Consensus* | [X-B-B-X-B-X] | Reference |
|------------|---------------|-----------|
| Vn†        | 302RKPSLAKKQRFRHHRNKRKGYSQ | 20        |
| Apo B      | 15ATRFKHLKRYTNYEAEESSS | 17        |
| Apo B      | 16GKALLKKTKNSEEFAAMSRY | 17        |
| Apo B      | 20DIQFVRKYAALGLKLPQQAND | 16, 17    |
| Apo B      | 21TSALTKKYRTENDIQALDD | 16, 17    |
| Apo B      | 24VKAQYKKKHRSITNPALV | 16, 17    |
| Apo B      | 25TSISGRQHRLVRSTAFVYTKN | 16, 17    |
| Fn         | 14GDTWRPHETGGYMLECVCL | 36        |
| Fn         | 17PWSPPRRAVTDAETTITIS | 36        |
| Fn         | 20ATPJRHRPPYPPNVGEEOIQI | 36        |
| Fn         | 24DQORHVKREEVVTGNSVE | 36        |
| TS         | 75LRQMKTRGTLLALERKDH | 37, 38    |
| LpL        | 27LCLSCRKNRCCNLLGYEINKVR | 39        |
| HTGL       | 22CLSLCSCKKGRCTLGGYHRQEP | 40, 41    |
| ECGF/FaFGF | 42LGNKYPKCLLYCNSNGYFLR | 42, 43    |
| THR        | 51LVRIGKHSRTYERNIKISM | 44        |
| NCAM       | 12FIWIKHKGDRVILKKDVRFIV | 45        |
| Apo E      | 205AWGERLRAMEMGStRDRL | 46        |
| GDN        | 128KAISSKNDIVTANAIVFK | 46        |

Table 1 represents the sequence similarity of basic regions in various heparin-binding proteins. The sequences are compared with the heparin-binding domains of vitronectin, apolipoprotein B-100, apolipoprotein E, and platelet factor 4.

*The consensus sequence (X-B-B-X-B-X) was derived from the usage frequency of basic (B) and nonbasic (X) residues in the data base (see Table 3).

†One-letter amino acid code: A=Ala, C=Thr, D=Asp, E=Glu, F=Phe, G=Gly, H=His, I=Ile, K=Lys, M=Met, N=Asn, P=Pro, Q=Gln, R=Arg, S=Ser, T=Thr, V=Val, W=Trp, and Y=Tyr.

This region diminishes heparin-binding to apo E, indicating that this region is utilized in the intact protein.14 These findings with synthetic peptides illustrate examples in which the consensus structures bind heparin and, together with the data in Tables 1 and 2 suggest that these sequence patterns are utilized by numerous proteins in polyanion binding. Thus, as shown below, the consensus sequences (Tables 1 to 4) may prove useful in identifying polyanion recognition sites in other proteins.

Predictions of Heparin-Binding Based on Sequence Inspection

Purpurin is a heparin-binding protein that functions in neuroretinal cell survival and adhesion.49 Its heparin-binding region has not been determined. However, inspection of its primary sequence (Table 5) reveals a single domain of high positive charge density that fits the consensus sequence [-X-B-B-X-B-X-B-X] given in Table 2. The homologous regions in other members of its gene family are also shown; these proteins exhibit reduced or no heparin-binding activity. The difference in their heparin reactivities may result in part from the nonconservation of positive charges at the -3 and +3 positions in the consensus; i.e., positions that show 60% and 100% usage by basic residues as determined from the data base of natural sequences summarized in Table 4.

We also examined the sequence of AT-III, which contains the consensus 130Y-R-K-A-N-K-S (Figure 1). This sequence may define a major region of heparin contact. Peterson et al.50 have shown that pyridoxyl-20 phenolic groups of a single critical lysine, lysine-125, blocks heparin-binding to antithrombin-III (AT-III); substantial pyridoxyl-20 labeling also occurred at lysines-133 and -136, i.e., residues within the consensus. Moreover, these residues are protected from pyridoxyl-20 labeling by heparin.60 More recently, Smith and Knauer61 isolated a heparin-binding fragment from AT-III containing the 130Y-R-K-A-N-K-S sequence. These data support, experimentally, a role for the 130Y-R-K-A-N-K-S...
sequence in heparin-binding. The corresponding homologous regions in glial-derived nexin (GDN) and heparin cofactor II (HC-II) are also shown (Figure 1). Like AT-III, these proteins utilize heparin as cofactor. GDN has two consensus structures, [X-B-B-B-X-B-X] and [X-B-B-X-B-X], given by residues 117 to 123 (L-K-K-N-K-A) and 126 to 131 (S-K-K-N-K-D), respectively. The K-K-N-K structure of GDN is identical to the four-residue stretch, 31 K-K-N-K, of the experimentally determined heparin-binding region of apo B-100 (see Table 1). HC-II contains the [X-B-B-B-X-B-X-X] consensus given by residues 183 to 189 (F-R-K-L-T-H-F). Other members of the SERPIN gene family, such as α_1-antitrypsin, α_1-antichymotrypsin, and C-I Inhibitor, which do not utilize heparin as cofactor, do not contain these consensus sequence elements. 52

Harper and Lobb 52 recently reported that reductive methylation of less than three lysines in acidic fibroblast growth factor (FGF) significantly reduced its heparin affinity, growth factor receptor affinity, and ability to stimulate mitogenesis in Balb/C 3T3 fibroblasts. Lys-118 was the primary site of dimethylation, and Lys-118 and -112 were the major sites of monomethylation. These residues reside in the predicted heparin-binding sequence 10 G-L-K-K-N-G-R-S-K (Table 2).

**Molecular Modeling of Heparin Binding**

Limited information is available on the solution conformations of heparin-binding domains. Recently we showed that the heparin-binding regions of apo E and apo B-100 defined by residues 129 to 169, 202 to 243, and 3345 to 3381, respectively, increase their helical and β-strand contents by 50% upon binding heparin. 57, 63 These regions fit the consensus [X-B-B-B-X-B-X]. The domain given by apo E 129 to 169 is known to form amphipathic α-helix upon lipid binding, 64 and both apo E 129 to 169 and apo B 3345 to 3381 show significant amphipathic α-helical periodicities as determined by Eisenberg analysis. 34, 57, 63 These findings suggest that heparin induces amphipathic α-helix in the apo E 129 to 169 and apo B 3345 to 3381 peptide
Figure 2. Helical wheel diagrams of the consensus sequence regions of various proteins.
Table 3. Distribution of Amino Acids in Heparin-binding Consensus [X-B-B-X-B-X-]

| Amino acid | X  | B  | B  | X  | B  | X  | Sum |
|------------|----|----|----|----|----|----|-----|
|            | -3 | -2 | -1 | +1 | +2 | +3 | -3 to +3 |
| E          | 0  | 0  | 0  | 0  | 2  | 2  | 2   |
| D          | 0  | 0  | 0  | 0  | 2  | 2  | 2   |
| K          | 1  | 1  | 2  | 0  | 4  | 1  | 28  |
| R          | 3  | 5  | 5  | 0  | 13 | 0  | 28  |
| H          | 0  | 3  | 2  | 0  | 2  | 1  | 8   |
| N          | 0  | 0  | 0  | 3  | 0  | 1  | 4   |
| Q          | 0  | 0  | 0  | 2  | 0  | 2  | 2   |
| C          | 0  | 0  | 0  | 0  | 2  | 4  | 4   |
| P          | 1  | 0  | 3  | 0  | 1  | 5  | 5   |
| S          | 1  | 0  | 0  | 1  | 0  | 2  | 2   |
| Y          | 2  | 0  | 2  | 0  | 2  | 0  | 4   |
| A          | 1  | 0  | 2  | 0  | 1  | 4  | 4   |
| G          | 2  | 0  | 2  | 0  | 1  | 5  | 5   |
| I          | 0  | 0  | 0  | 0  | 0  | 1  | 1   |
| L          | 1  | 1  | 0  | 1  | 0  | 2  | 5   |
| M          | 1  | 0  | 0  | 0  | 0  | 1  | 2   |
| F          | 1  | 0  | 0  | 0  | 0  | 1  | 2   |
| T          | 1  | 0  | 0  | 2  | 0  | 1  | 4   |
| W          | 1  | 0  | 0  | 0  | 0  | 1  | 1   |
| V          | 1  | 0  | 1  | 0  | 1  | 3  | 3   |

| Acidic residues | 0  | 0  | 0  | 0  | 0  | 4  | 4   |
| Basic residues  | 4  | 18 | 19 | 0  | 19 | 2  | 62  |
| Aromatic residues | 4  | 0  | 2  | 0  | 1  | 7  |      |

The residues distributions were compiled from the natural amino acid sequences of heparin-binding proteins of Table 1.

regions. As is shown in Figure 2, helical wheel diagrams of these peptide regions segregate the basic residues primarily to one side of the helical face, forming a region of high positive charge density. The [X-B-B-X-B-X-B-X-] motif ensures the segregation of the basic residues in the consensus to one side of the helical face. In addition, basic residues flanking the region of the consensus may also contribute to the charge density. For proteins assuming α-helical changes upon heparin-binding, the [X-B-B-X-B-X-B-X-] may serve as the primary nucleation site for heparin interaction. Subsequent to the structural change, the polyanion may "lock" or stabilize the peptide in this configuration. Figure 2 also shows helical wheel projections for purpurnin, retinol binding protein, AT-III, and FGF. However, the conformations that these regions assume upon heparin-binding are not known. One possible explanation for the reduced heparin affinity in retinol binding protein relative to purpurnin is a significant decrease in positive charge density within the region of the consensus. Based on the X-ray crystal structure of α-antitrypsin, Carroll et al.65 modeled the heparin-binding region of AT-III as α-helical. The AT-III insertion region used in the study includes the consensus sequence [G]L-Y-R-K-A-N-K-S (see Table 2 and Figure 1), and it has amphipathic periodicity by the Eisenberg analysis.64 Based on helical wheel diagrams,25 acidic FGF shows potential to form α-helix. Chemical modification of lysine-112 and lysine-118 by reductive methylation25 might possibly destabilize the charge interaction with heparin and hence the helical conformation.

Heparin binds amphiphilic β-strand structure in apo E.63 The sequences involved are R-L-A-R-H-M and R-T-R-P-R-L, which consist of alternating basic and nonbasic residues of the consensus [-X-B-B-X-B-X-]. Beta-structure is also induced by heparin in model peptide copolymers having alternating lysine and tyrosine residues.66 Thus, heparin may increase the β-strand and α-helical character depending on the precise organization of basic and nonbasic residues in natural peptide sequences.

Polynians may also bind to sites on proteins without inducing significant changes in protein secondary structure. Proteins may contain exosites contoured to accommodate heparin of specific size and charge. Figure 3 illustrates one potential representation of the binding of a heparin octasaccharide to the heparin-binding region of Vitronectin. The heparin and VN structures were individually minimized by the Discover force field82 to locate a stable conformation of each molecule. The two molecules were manually docked, and molecular dynamics simulation followed by energy minimization of the docked structures refined the fit without significant changes to the original unminimized docked structure of heparin or VN. As shown in Figures 3A to 3D, the VN domain forms a folded charged pocket into which the heparin octasaccharide fits. The cleft, formed by the twisting of a series of β-bends from the carboxy-terminal end, is capped by the amino-terminal helical portion of the VN structure. The [-X-B-B-X-B-X-] and [-X-B-B-X-B-X-B-X-] elements of the domain form distinct areas of contact with heparin that wrap around and fold over the octasaccharide, yielding a structure complementary to charge and contour. Thus, heparin molecules not having the correct complementary structures may not bind. In addition to the

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**Figure 3.** A molecular model of the interaction of a heparin octasaccharide with the heparin-binding region of vitronectin (VN). The structure of VN represents residues 342 to 369 containing the amino-terminal [-X-B-B-X-B-X-] and carboxyl terminal [-X-B-B-X-B-X-B-X-] consensus sequence elements. A. This view represents a look down the z-axis of the two interacting molecules. As shown, VN (white) forms a structure that wraps around the heparin molecule (red). This space-filling view shows the complementarity of surface densities for the two molecules. B. A side view of the two interacting molecules. The carbohydrate ring of heparin is shown in blue and the sulfates and oxygen of the hydrophilic side chains in yellow and red, respectively. The α-carbon backbone of VN is shown in white and the nitrogens of its side chains in blue. C. The same view as in B but with the added surface densities. The surface densities for VN are shown by white dots and those for heparin by red dots. Note the complementarity of the surface densities. D. A 180° rotation of the view shown in C. Note the finger-like projections of the VN side chains that wrap around to cradle the heparin molecule. The color codes for the various atoms in views B to D are the same as in A.
Table 4. Distribution of Amino Acid Residues in Heparin-binding Consensus [X-B-B-X-B-X-B-X]

| Amino acid | X-4 | B-3 | B-2 | B-1 | X+1 | X+2 | B+3 | X+4 | Sum -4 to +4 |
|------------|-----|-----|-----|-----|-----|-----|-----|-----|-------------|
| E          | 0   | 0   | 0   | 0   | 0   | 2   | 0   | 0   | 2           |
| D          | 1   | 0   | 0   | 1   | 0   | 0   | 1   | 3   |             |
| K          | 0   | 7   | 16  | 11  | 0   | 0   | 17  | 2   | 53          |
| R          | 4   | 8   | 11  | 5   | 0   | 0   | 9   | 3   | 40          |
| H          | 0   | 1   | 1   | 0   | 0   | 2   | 1   | 6   |             |
| N          | 0   | 2   | 0   | 1   | 5   | 0   | 1   | 9   |             |
| Q          | 0   | 0   | 0   | 1   | 3   | 0   | 1   | 6   |             |
| C          | 0   | 0   | 0   | 0   | 1   | 0   | 0   | 1   |             |
| P          | 2   | 1   | 0   | 2   | 1   | 0   | 0   | 6   |             |
| S          | 2   | 1   | 0   | 2   | 1   | 0   | 3   | 9   |             |
| Y          | 1   | 2   | 0   | 2   | 1   | 0   | 2   | 9   |             |
| A          | 1   | 1   | 0   | 1   | 4   | 0   | 0   | 10  |             |
| G          | 2   | 0   | 0   | 3   | 3   | 6   | 0   | 16  |             |
| I          | 3   | 1   | 0   | 1   | 4   | 3   | 0   | 15  |             |
| L          | 5   | 3   | 0   | 1   | 5   | 3   | 0   | 19  |             |
| M          | 1   | 0   | 0   | 0   | 0   | 0   | 0   | 4   |             |
| F          | 0   | 1   | 0   | 0   | 1   | 0   | 0   | 2   |             |
| T          | 5   | 0   | 0   | 0   | 0   | 1   | 0   | 6   |             |
| W          | 1   | 0   | 0   | 1   | 1   | 0   | 0   | 3   |             |
| V          | 0   | 0   | 0   | 1   | 2   | 1   | 0   | 5   |             |
| Acidic residues* | 1 | 0 | 0 | 0 | 1 | 2 | 0 | 5 |             |
| Basic residues* | 4 | 16 | 28 | 17 | 0 | 0 | 28 | 6 | 104         |
| Aromatic residues* | 2 | 3 | 0 | 3 | 3 | 1 | 0 | 2 | 13          |

The residue distributions were compiled from the natural amino acid sequences of heparin-binding proteins of Table 2.

*The percent composition of basic, acidic, and aromatic residues at each position within the consensus is given by:

\[ [X-B-B-X-X-B-X-B-X] \]

\[ \% \text{Basic} = 14 \quad 57 \quad 100 \quad 61 \quad 0 \quad 100 \quad 21 \]

\[ \% \text{Acidic} = 3 \quad 0 \quad 0 \quad 3 \quad 7 \quad 0 \quad 3 \]

\[ \% \text{Aromatic} = 6 \quad 11 \quad 0 \quad 11 \quad 1 \quad 3 \quad 0 \]

Table 5. Proposed Heparin Recognition Region of Purpurin: Comparison to Homologous Regions in Other Sequence-related Proteins

| Proteins          | [X-B-B-X-B-X-B-X] | Reference |
|--------------------|-------------------|-----------|
| Purpurin           | 27PKRYAGKWYALAKK  | 49        |
| Retinol-binding protein | 26KARFSGTWYAMAKK   | 49        |
| α-Lactoglobulin    | 12SRIYGYKNLAI    | 85        |
| β-Lactoglobulin    | 12QKVAGTWAYSLAM  | 85        |

consensus regions, the folding is such that basic residues outside the consensus are brought into contact with the hydrophilic side chains of heparin. The presence of these domains in Vn and possibly other proteins may facilitate the interaction of neighboring amino acid residues with the heparin molecule. In this way, different heparin structures may bind different heparin-binding proteins. In addition to electrostatic binding, the model shown in Figure 3 implies attractive forces due to van der Waals interactions and formation of intermolecular hydrogen bonds. Table 6 lists some of the quantitative parameters of the model.

The Vn sequence contains both heparin-binding consensus sequence elements in series. In lipoprotein lipase (LPL) and endothelial cell growth factor (ECGF)/acidic FGF, for example, the consensus sequence elements are not close together within the primary sequence. However, two or more of these regions may come together by folding of the protein structure to form a topological domain or crevice into which the heparin molecule binds. In contrast, other proteins may not exhibit a particular specificity for heparins differing in size, charge, and carbohydrate composition, depending on the organization of their polyanion recognition domains. Such interactions should not be de-emphasized in importance as they may serve a biological function. These studies point out the unique sequence commonalities and conformational orga-
organizations specifying polyanion recognition among proteins of diverse biological function.

**Discussion**

**Significance to the Pathogenesis of Atherosclerosis**

The purpose of this study was to obtain structural insight into the interactions of GAG with proteins, as these molecules are involved in numerous cardiovascular processes, including lipoprotein-proteoglycan interactions in the arterial wall,\(^5\) cell substratum adhesion and spreading,\(^4\) regulation of smooth muscle cell proliferation,\(^5,6\) hemostatic processes,\(^1,2,3\) and reovascularization.\(^1,12\) Our studies have focused primarily on the binding of LDL with heparin as a model of lipoprotein-arterial wall GAG interactions.

A limited number of regions in apo E and apo B-100 bind heparin.\(^5-10\) The LDL receptor-binding region in apo E\(^24\) corresponds to a heparin-binding region\(^18,19\) and is structurally similar to the heparin-binding region defined by residues 3352 to 3378 of apo B-100.\(^16,17\) Studies with monoclonal antibodies to apo B-100 that block LDL binding to the receptor have been interpreted as a single domain for receptor recognition.\(^5,6,71\) In contrast, studies on the interaction of heparin with LDL show that there are five to seven heparin contact sites on the LDL surface,\(^5\) and approximately this number of heparin-binding peptides have been purified from apo B-100.\(^16,17\) It is known that heparin releases LDL from cell receptors.\(^71\) Heparin may inhibit the binding of LDL to the receptor by binding to the region of apo B-100 involved in receptor-recognition, possibly residues 3352 to 3378, which resemble the receptor-binding sequence of apo E. Alternatively, heparin may bind to other sites on the LDL surface that inhibit receptor binding. Presently, it is not known what effect increased circulating levels of endogenous GAG\(^22\) have on LDL catabolism.

We previously showed that proteolysis of the LDL surface with trypsin significantly diminished receptor-binding without appreciably affecting heparin binding.\(^73\) It is possible that one or more mutations affecting the receptor-binding region of apo B-100 that inhibits LDL receptor binding may not affect the ability of the particle to bind arterial proteoglycan. Such a molecule would be particularly atherogenic since it would not be removed from the circulation by the liver via the LDL receptor pathway. Such lipoproteins may complex with GAG and be taken up by macrophages to form foam cells. During progression of atherosclerosis, the changes in the GAG composition of the arterial wall that are known to occur may further potentiate the interaction with LDL particles.\(^24\) Polymorphisms in the protein and DNA structure of apo B-100 have been detected,\(^75-78\) and in two recent studies, structural mutations in LDL apo B-100 have been implicated in primary moderate hyperlipoproteinemia and atherosclerosis.\(^77,78\) These observations may be explained by mutations that alter receptor binding but not the lipoprotein's interaction with arterial wall glycosaminoglycans. Stinizovs et al.\(^79\) recently reported that heparin inhibits LDL accumulation in rabbit aorta, suggesting the possibility that heparin occupancy of the GAG-binding domains on the LDL surface may block LDL reactivity with arterial

| Consensus sequence | Residue number | Amino acid residue | Interatomic distance (Å) | Heparin side chain |
|--------------------|----------------|-------------------|-------------------------|--------------------|
| X                  | 342            | R                 | 1.99                    | O-sulfate          |
| B                  | 346            | A                 |                         |                    |
| B                  | 347            | K                 |                         |                    |
| B                  | 348            | K                 | 2.26                    | carboxylate        |
| X                  | 349            | Q                 |                         |                    |
| B                  | 350            | R                 |                         |                    |
| X                  | 351            | F                 |                         |                    |
| B                  | 352            | F                 | 4.12                    | carboxylate        |
| B                  | 353            | H                 | 6.46                    | O-sulfate          |
| X                  | 354            | R                 | 10.05                   | O-sulfate          |
| B                  | 355            | N                 |                         |                    |
| B                  | 356            | R                 | 7.19                    | O-sulfate          |
| B                  | 357            | K                 | 2.01                    | O-sulfate          |
| X                  | 358            | G                 |                         |                    |
| X                  | 359            | Y                 |                         |                    |
| B                  | 360            | R                 | 2.93                    | O-sulfate          |
| X                  | 361            | S                 |                         |                    |
| B                  | 362            | R                 | 9.41                    | O-sulfate          |
| B                  | 363            | H                 | 3.18                    | O-sulfate          |
| X                  | 364            | R                 | 3.84                    | O-sulfate          |

These interatomic distances are representative of one of the sampled geometries from the molecular dynamics trajectory. The interactions listed yield minimal energy contributions.
proteoglycan. Ye et al. reported that the lysine residues on apo B-100 are involved in an interaction with the plasminogen-like domains (Kringle 4) on apo(a). These researchers proposed that the association of LP(a) with lysine-rich regions in apo B-100 may diminish the interaction of LDL with the LDL receptor, as reported for apo(a), and direct the particles into other catabolic pathways. Such complexes, however, may still bind GAG. In this regard, Kostner et al. reported that LP(a) and LDL readily formed complexes with GAG that caused massive cholesteryl ester accumulation in mouse peritoneal macrophages. Most recently, Horn-Brahi et al. reported a role for proteoglycan in the binding of LDL to cultural arterial smooth muscle cells and proposed that the LDL-proteoglycan interaction leads to lipoprotein cholesterol accumulation in vascular tissues. Carneiro et al. showed that three of nine synthetic peptides corresponding to different hydrophilic regions of apo B-100 inhibited the binding of LDL to an intermedia-media chondroitin sulfate proteoglycan. Two of the three peptides, P-2 (3359 to 3377) and P-11 (2106 to 2121), represent apo B-100 heparin-binding domains, and the third, P-1 (4230 to 4254), contained a heparin-binding consensus sequence. With these points in mind, the heparin-binding domains of apo B-100 should be considered important regions that may affect LDL metabolism in the arterial wall.

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