Design of Peptides with High Affinities for Heparin and Endothelial Cell Proteoglycans*

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Proteoglycan-binding peptides were designed based on consensus sequences in heparin-binding proteins: XBBXBX and XBBBXXBX, where X and B are hydrophilic and basic residues, respectively. Initial peptide constructs included (AKKARA)$_n$ and (ARKKAAKA)$_n$ (n = 1–6). Affinity coelectrophoresis revealed that low $M_r$ peptides (600–1300) had no affinities for low $M_r$ heparin, but higher $M_r$ peptides (2000–3500) exhibited significant affinities ($K_d$ = 50–150 nM), which increased with peptide $M_r$. Affinity was strongest when sequence arrays were contiguous and alanines and arginines occupied hydrophilic and basic positions, but inclusion of prolines was disruptive. A peptide including a single consensus sequence of the serglycin proteoglycan core protein bound heparin strongly ($K_d$ = 200 nM), likely owing to dimerization through cysteine-cysteine linkages. Circular dichroism showed that high affinity heparin-binding peptides converted from a charged coil to an $\alpha$-helix upon heparin addition, whereas weak heparin-binding peptides did not. Higher $M_r$ peptides exhibited high affinities for total endothelial cell proteoglycans ($K_d$ = 300 nM), and ~4-fold weaker affinities for their free glycosaminoglycan chains. Thus, peptides including concatamers of heparin-binding consensus sequences may exhibit strong affinities for heparin and proteoglycans. Such peptides may be applicable in promoting cell-substratum adhesion or in the design of drugs targeted to proteoglycan-containing cell surfaces and extracellular matrices.

Proteoglycans (PGs)‡ are composed of a core protein to which are covalently attached one or more sulfated glycosaminoglycans (GAGs). PGs are ubiquitous components of cell surfaces and the extracellular matrix, and their GAG chains contribute to myriad biological functions, such as modulation of enzyme activities, regulation of cell growth, and control of assembly of the extracellular matrix (1). PGs are thus potential targets for therapeutic intervention. For example, heparin antagonists are needed to take the place of protamine, a heterogeneous, sometimes toxic protein mixture commonly used to neutralize the anticoagulant activity of heparin in humans (2, 3); in the design of drugs to be targeted to PG-rich tissues, such as cartilage (4); and to be used to promote cell adhesion in a variety of situations, e.g. by promoting binding of cells that express abundant amounts of PGs, such as endothelial cells (5), to synthetic vascular graft surfaces. To develop a rationale for the design of such reagents, it is useful to examine known features of protein structure required for high affinity interactions with GAGs. Thus, analysis of the structural features of many known heparin- and heparan sulfate (HS)-binding proteins has revealed the presence of conserved motifs, through which GAG binding has been postulated to occur. Cardin and Weintraub (6) identified two clusters of basic charge in known heparin-binding proteins in which amino acids tend to be arranged in the patterns XBBXBX or XBBBXXBX, where $B$ represents an amino acid with basic charge, usually arginine or lysine, and $X$ represents an uncharged or hydrophobic amino acid. Molecular modeling of these consensus sites predicts the arrangement of amino acids into either $\alpha$-helices or $\beta$-strands. This allows for the clustering of noncontiguous basic amino acids on one side of the helix, thus forming a charged domain to which GAGs could bind. Indeed, for some but not all of the heparin-binding proteins, disruption of the heparin-binding consensus sequences hinders heparin binding. For example, chemical modification of the heparin-binding consensus site in thrombospondin (7) or site-directed mutagenesis of a heparin-binding sequence in fibronectin (8) eliminates or diminishes heparin binding affinity. Others have proposed a necessary distance of approximately 20 Å between basic amino acids for heparin binding, regardless of protein tertiary structure (9).

To date, few attempts have been made to use these concepts regarding the structural specificities of GAG-protein interactions to develop families of high affinity GAG- or PG-binding peptides. Thus, here we describe the design and characterization of high affinity heparin- and EC PG-binding peptides that were modeled from the proposed heparin-binding consensus sequences of native heparin-binding proteins.

**EXPERIMENTAL PROCEDURES**

**Peptide Synthesis**—Peptides were synthesized and purified by the University of Virginia Biomolecular Research Facility (Charlottesville, VA) or by Genosys Biotechnologies (The Woodlands, TX). Peptides were synthesized by standard solid phase synthesis using Fmoc (N-$\beta$-fluoron- nyl)methoxycarbonyl) chemistry. Peptide molecular weight was verified by mass spectroscopy, and purity (>90%) was analyzed by high pressure liquid chromatography.

**Preparation of Radiolabeled Heparin**—Whole heparin from pig intesti...
tinal mucosa (Sigma) was tyramine end-labeled and radiolabeled with Na$_{251}$I (Amersham Pharmacia Biotech) to an average specific activity of $\sim 1.0 \times 10^5$ cpm/$\mu$g as described (10). Radiolabeled heparin was fractionated on Sephadex G-100 (Bio-Rad), and the final $\sim 12$% of material to elute was retained as the low $M_c$ material of $\sim 6000$ (11, 12).



**Extraction of Binding of Heparin and Human Umbilical Vein Endothelial Cell (HUVEC) PGs to Peptides**—Binding of radiolabeled heparin and HUVEC PGs to peptides was studied by ACE as detailed elsewhere (13), because the heparin-protein binding affinities revealed by ACE match reasonably well with those obtained by other well established quantitative techniques for measuring binding interactions (14–17). Briefly, peptides were dissolved in running buffer, 50 mM MOPS (Sigma)/125 mM sodium acetate, pH 7.0, and serially diluted in running buffer at 2$^\times$ concentrations. Peptides were then mixed 1:1 with 2% agarose/1% CHAPS (Roche Molecular Biochemicals) and loaded into wells of a 1% agarose gel. Radiolabeled heparin or HUVEC PGs were then loaded in a slot on the anode side of the gel and electrophoresed through the peptide-containing wells, toward the cathode. Gels were dried, and PG mobility was measured with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA) by scanning each protein lane and determining the relative radioactivity content per 88-$\mu$m pixel through the length of the lane. Retardation coefficient ($R$) measurements, binding isotherm curve fittings, and apparent $K_d$ value determinations were calculated as detailed previously (10, 13).

**Binding of radioactive peptides to enzymatically or chemically degraded PGs (see below) was carried out by ACE as detailed, except that PG samples included 6 M urea to denature any residual enzymes.**

**Cell Culture—**HUVEC were isolated as detailed elsewhere (18) and were used up to passage seven. Cells were cultured on 0.2% gelatin-coated tissue culture flasks in normal culture medium composed of medium 199 (Life Technologies, Inc.), 10% fetal bovine serum (Medi-tech Inc.), 50 g/ml endothelial cell growth supplement isolated from bovine hypothalami as described (19), 50 mM hydrocortisone, 0.5 mg/ml bovine serum albumin, 10 mM $\text{N}_2\text{H}_4$, and 0.1 mM penicillin-streptomycin, and 0.1% fungizone.

**Radiolabeling and Isolation of Total HUVEC PGs and GAGs—**Exponentially growing, subconfluent HUVECs were labeled with 35 cpm/$\mu$mol [35$\text{S}]$Na$_2$SO$_4$ (ICN Pharmaceuticals, Costa Mesa, CA) in normal culture medium minus heparin for 12 h. Culture medium and cell layers were harvested separately. After removal of the cell layer by centrifugation, the medium, cells, and PGs were washed with 2.0 ml of phosphate-buffered saline plus Ca$^{2+}$-Mg$^{2+}$. Media and rinses were pooled and brought to 6 M urea, 10 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 5 mM N-ethylmaleimide, 50 mM 6-amino-caproic acid, 5 mM benzamidine, and 1 $\mu$mol/ml penicillin. Samples were stirred for 15 min at room temperature and then centrifuged at 10,000 rpm for 30 min to remove insoluble materials.

The cell layer was washed 2.0 ml of extraction solution, 6 M urea, 100 mM NaCl, 0.2% Triton X-100, 50 mM Tris-HCl, pH 7.0, and protease inhibitors as described above. Cells were scraped off the dishes, and the extracts were pooled and stirred for 5 min at room temperature and then centrifuged as described above.

**Sample Preparation for Heparin Binding under reducing conditions.** Thus, after serial peptide dilution, $\beta$-mercaptoethanol was added at 5% to each peptide sample, and these were mixed 1:1 with 2% agarse/1% CHAPS/5% $\beta$-mercaptoethanol and added to the ACE gel sample wells as usual.

**Binding analysis of peptides to enzymatically or chemically degraded PGs (see below) was carried out by ACE as detailed, except that PG samples included 6 M urea to denature any residual enzymes.**

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with the highest probability of occurrence in each basic position in the heparin-binding consensus sequences of native heparin-binding proteins (6). When single copies of either sequence were tested for heparin binding by ACE, no affinities were detected. In contrast, peptides containing two copies of the consensus sequence exhibited weak but detectable affinities for heparin (<6 µM), and peptides of higher molecular weight containing 4–6 copies of a consensus sequence showed a marked increase in heparin binding affinity (40–150 nM) (Fig. 1). The heparin binding affinity of both the 6-mer and 8-mer tandem repeat peptides reached a plateau as peptide length approached 30 amino acids ((AKKARA)5, Kd > 740 nM (p < 0.01), affinity was decreased (Table I)).

To define the sequence and conformational features of the tandem repeat peptides that confer their high affinity heparin binding characteristics, peptides containing variants of one of the consensus sequences first tested, (AKKARA)5, were synthesized. These included those in which alanines were replaced by glycine in all the hydrophilic positions ((ARKKAAKA)3, (AKKARA)3, (ARKKAAKA)4, (ARRRAARA)3, (ARRAKA)3, (AKAAKKRA)3, (GRKKGGKG)3, (LRKKLGKR)3, (AKRKKAAKA)3, (TRKKLGKI)3, (AKAAKKRA)3, (ARKKAAKAARKKPAKAARKKAAKA)2, (ARKKAAKARKKAKARKKAAKA)2, (ARKKAAKAAAAAARKKAAKA)2, (ARKKAAKAAAAAARKKAAKAARKKAAKA)5) displayed similar affinities (Kd ≈ 100 and 50 nm, respectively; Table I). The heparin binding affinity of both the 6-mer and 8-mer tandem repeat peptides reached a plateau as peptide length approached 30 amino acids ((AKKARA)5, Kd ≈ 90 nM; (ARKKAAKA)5, Kd ≈ 40 nM). Larger peptides ((AKKARA)6 and (ARKKAAKA)6) displayed similar affinities (Kd ≈ 100 and 50 nm, respectively; Table I).

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#### Table I: Heparin binding affinity of peptides containing heparin-binding consensus sequences

| Peptide sequence | M, Kd ± S.D. |
|------------------|--------------|
| XBBXX tandem repeats |  |
| AKKARA | 644 | Not detectable |
| (AKKARA)2 | 1270 | 40,000 ± 18,000 |
| (AKKARA)3 | 1895 | 1,902 ± 719 |
| (AKKARA)4 | 2520 | 174 ± 19 |
| (AKKARA)5 | 3146 | 94 ± 41 |
| (AKKARA)6 | 3770 | 104 ± 32 |
| (ARRAKA)3 | 1979 | 900 ± 170 |
| XBBXX tandem repeats |  |
| (ARKKAAKA)2 | 843 | Not detectable |
| (ARKKAAKA)3 | 1668 | 6,200 ± 3,000 |
| (ARKKAAKA)4 | 2493 | 135 ± 54 |
| (ARKKAAKA)5 | 3318 | 42 ± 15 |
| (ARKKAAKAPKA)2 | 4143 | 51 ± 11 |
| (ARRRRAARA)3 | 2745 | 72 ± 22 |
| (ARKKAAKAARKKPAKAARKKAAKA)2 | 2493 | 132 ± 93 |
| XBBXX tandem repeats with hydrophobic position modifications |  |
| (ARKKAAKA)2 | 2878 | 75 ± 41 |
| (AKKARA)3 | 2325 | 200 ± 98 |
| (ARKKAAKA)4 | 2959 | 105 ± 37 |
| (LRKKLGKR)3 | 2794 | 737 ± 350 |
| (ARRRAARA)3 | 2571 | 360 ± 127 |
| (ARKKAAKAARKKPAKAARKKAAKA)2 | 2519 | 730 ± 340 |
| (ARKKAAKARKKAKARKKAAKA)2 | 3351 | 450 ± 95 |
| (ARKKAAKAAAAAARKKAAKAARKKAAKA)5 | 3062 | 254 ± 137 |
| Native or modified serglycin sequences |  |
| YPARRARYQWVRCKP | 1948 | 187 ± 54 |
| YPTQRRARYQWVRCP | 1936 | 417 ± 170 |
| YPARRARYQWVRKP | 1918 | 37,000 ± 6,700 |
| AAARRARAAARKA | 1482 | 72,000 ± 60,000 |

*pe < 0.01 versus (AKKARA)5.

p < 0.01 versus (AKKARA)6.

p < 0.05 versus (ARKKAAKA)5.

p < 0.01 versus (ARKKAAKA)6.
between adjacent consensus sequences were altered. Both increasing (ARKKAARKKAARKKAARKKAAKA) and decreasing (ARKKAARKKAAARKKAAKAA) the distance between consensus sequences resulted in decreased heparin binding affinity ($K_d > 250$ and 450 nM, respectively). Inclusion of prolines also decreased the heparin binding affinity, the degree of which was influenced by their position and number. Thus, the heparin binding affinity decreased to 360 nM when prolines were present in each tandem repeat in place of alanines (AAARRARAAAARAKA). A 350-fold decrease in heparin binding affinity ($K_d < 360$ nM) was observed when a single proline was substituted in the center of a series of three heparin-binding consensus sequences (ARKKAAARKKAAARKKAAKAA, $K_d = 6000$ nM; Table I).

Other peptides synthesized and studied include sequences native to the mouse (YPARRARYQWVRCKP (●) or human (YTPTQARYGWVRNPCP (○)) SG PG core proteins were determined from ACE gel electrophoretograms as detailed under “Experimental Procedures.” SG peptides displayed relatively strong affinities for heparin ($K_d = 200$ and 900 nM for the mouse and human peptides, respectively), in comparison to peptides of similar size that contain multiple repeats of heparin-binding consensus sequences (e.g. (AKKARA)$_n$, $K_d = 2000$ nM, and (ARKKAARKKAARKKAAKAA), $K_d = 6000$ nM; Table I). Peptide AAARRAARAAARRAKA (●) displayed negligible heparin binding affinity ($K_d > 75$ μM), indicating the importance of the nonbasic residues to heparin binding. YPARRARYQWVRCKP-heparin binding in the presence of β-mercaptoethanol (YPARRARYQWVRCKP + β-mercaptoethanol (×)) was decreased by over 20-fold ($K_d = 4$ μM). Replacement of cysteine by alanine in the mouse SG peptide (YPARRARYQWVRKA○) further reduced heparin binding affinity ($K_d < 36$ μM).

Peptides that displayed weak ((AKKARA)$_2$), moderate ((AKKARA)$_4$), and strong ((AKKARA)$_5$ and (AKKARA)$_6$) heparin binding affinities were analyzed by CD to characterize their degree of α-helical contents and propensities to form an α-helix. All peptides exhibit very similar spectra with peaks at 195 and 216 nm and a crossover at 210 nm (for example, see Fig. 3, (AKKARA)$_6$, 1:0 (●)), and Fig. 4, (AKKARA)$_2$, 1:0 (●)). These spectra are indicative of an extended charged coil conformation that was previously reported for charged poly-L-lysines and poly-L-arginines (26).

Intrinsic CD of the peptides shows that they do not adopt α-helical conformations. To explore the conformational repertoire of the peptides and to record CD spectra for the α-helical conformations, peptides were analyzed by CD in the presence of the nonpolar solvent TFE. Nonpolar solvents are known to increase the degree of α-helicity of a peptide in solution by enhancing hydrogen bonding and electrostatic interactions (27). CD of (AKKARA)$_6$ at 0.1 mg/ml containing 10, 20, 30, 40, and 50% TFE (v/v) was measured. At TFE concentrations >30%, with an apparent maximal effect induced at 40% TFE, the peptide assumes an α-helical conformation with classic α-helical peaks at 206 and 220 nm and a crossover at 197 nm (data not shown).

The CD spectra of (AKKARA)$_6$ recorded in the presence of increasing amounts of heparin (Fig. 3) demonstrate that a change from a charged coil conformation displayed in the absence of heparin (1:0) occurs upon heparin addition (1:0.25, 1:0.50, and 1:1). Heparin induces a similar α-helical conformation at a 1:1 peptide:heparin ratio that was obtained in the presence of >30% TFE, with classic α-helical peaks at 190, 207, and 222 nm. Excess heparin (1:2 or 1:4 (●)) disrupts this interaction, and the spectra resemble that of a protein in a random coil conformation. Spectra are heparin and/or blank (water) corrected.

CD—The intrinsic structural properties of the peptides were explored using CD spectroscopy. Short peptides of known heparin-binding proteins containing heparin-binding consensus sequences have previously been shown to fold into α-helical conformations. In doing so, the basic amino acids locate to one face of the helix and thus are potentially exposed for binding. Peptides that displayed weak (AKKARA)$_2$, moderate (AKKARA)$_4$, and strong (AKKARA)$_5$ and (AKKARA)$_6$ heparin binding affinities were analyzed by CD to characterize their degree of α-helical contents and propensities to form an α-helix. All peptides exhibit very similar spectra with peaks at 195 and 216 nm and a crossover at 210 nm (for example, see Fig. 3, (AKKARA)$_6$, 1:0 (●), and Fig. 4, (AKKARA)$_2$, 1:0 (●)). These spectra are indicative of an extended charged coil conformation that was previously reported for charged poly-L-lysines and poly-L-arginines (26).

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α-helical conformation of a polypeptide in solution has been reported previously (26).

This same heparin effect is not obtained for the weak heparin-binding peptide (AKKARA)$_2$ (Fig. 4). In the absence of heparin (1:0), the peptide assumes a similar charged coil conformation as that observed for (AKKARA)$_6$ but fails to display α-helical character in the presence of heparin (1:0.25, 1:0.50, 1:0.75, or 1:1).

**Peptide-PG Interactions**—The interactions between consensus sequence peptides and PGs were also examined. For these experiments, total PGs were isolated from HUVEC cultures, because HUVECs have been shown to express a variety of types of HS and CS PGs, including, for example, syndecans, perlecan, glypican, and biglycan (28, 29). Thus, cell layer-associated and secreted [35S]SO$_4$-radiolabeled PGs were purified by extraction of HS and CS PGs, including, for example, syndecans, perlecan, glypican, and biglycan (28, 29). Then, cell layer-associated and secreted [35S]SO$_4$-radiolabeled PGs were purified by extraction with urea, and those PGs retained on DEAE after a 0.1 M NaCl rinse were studied for their binding to (ARKKAAKA)$_4$ by ACE (Fig. 5A, EC PGs). This peptide exhibited significant affinity for secreted HUVEC PGs, although the average affinity was somewhat weaker than that exhibited by the peptide for heparin (PG $K_d \approx 300$ nM; heparin $K_d \approx 50$ nM). Similar affinities were obtained for cell layer-associated PGs (data not shown). Inspection of ACE gels in which secreted PGs were fractionated through peptides demonstrated the presence of at least two populations of PG evident as two distinct bands of radiolabeled material migrating through the peptide lanes with different mobilities (Fig. 5A, EC PGs). This difference in migration rate could indicate heterogeneity of the PG in size or charge. In contrast to the heterogeneity seen in Fig. 5A, Fig. 5B shows that heparin migrates as a single broad band of radiolabeled material.

Thus, to ascertain which GAG chains, as well as which PG component (i.e. core protein, GAG chains, or both), were responsible for peptide binding, total HUVEC PGs were subjected to various chemical and enzymatic degradations. Samples were then tested for their ability to bind to (ARKKAAKA)$_4$, PGs in which HS GAGs were chemically degraded by nitrous acid or enzymatically degraded by heparinase I were able to maintain comparable affinity for the peptide as was displayed by the total PG sample (Fig. 5A, EC PGs/NA, and Fig. 6). PGs in which CS GAG chains were digested with chondroitinase ABC were also able to maintain comparable affinity for the peptide. Release of GAG chains from cores by borohydride reduction resulted in a 3–4-fold diminished affinity (Fig. 6).

**DISCUSSION**

The goal of this study was to design high affinity heparin- and PG-binding peptides; the strategy we used was to incorporate into their structure copies of sequences proposed to bind heparin in native proteins. Our approach was also based on the fact that truncation of peptide structure without loss of activity...
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can sometimes be achieved by constraining or manipulating peptide conformation (30). In the case of apolipoprotein E and apolipoprotein B-100, heparin-binding sites are believed to form α-helices upon heparin binding, and molecular modeling illustrates that basic amino acids in the binding sites align to one side of the helix to form a region of high positive charge through which heparin binding occurs (6). Thus, in our design of heparin-binding peptides, we also incorporated structural features conducive to stable α-helicity.

In our initial experiments, families of peptides were synthesized that contained single or multiple copies of heparin-binding consensus sequences. When their heparin binding was examined by ACE, peptides containing single sequences showed no measurable affinity for heparin. This result is as expected because peptides carrying single heparin-binding sequences found in native proteins often fail to display significant heparin binding (24), but they may contain multiple consensus sequences that come into proximity upon protein folding or multimerization, thereby enhancing heparin binding through cooperativity (31). In contrast, the affinity of peptides (AKKARA)ₙ or (ARKKAAKA)ₙ ranged from weak (K_d = 6–40 μM) at n = 2 to strong (K_d = 50–100 nM) at n = 3–6. These latter affinities are in the range of those displayed by heparin-binding peptides of the type (AKKARA)₂, failed to undergo any conformational change. In contrast, a peptide that displayed weak heparin binding, (AKKARA)₆, underwent a simple strategy to greatly enhance the affinity of peptides for heparin, SG, cerebroglycan (with PRRLRL) (33), and perlecan (with TRRFRD) (34) are among the few PGs that contain heparin-binding consensus sequences on their core proteins. Interestingly, the SG core protein, which carries many heparin chains, migrates at twice its predicted molecular weight on PAGE gels under reducing conditions (35), suggesting dimerization. This could result from GAG chains of one PG binding to the core protein of another or from core-core associations through disulfide bonding. The potential physiological function of such PG-PG interactions remains to be explored.

Additional consensus sequence peptides were designed to determine other aspects of peptide structure important to heparin binding. Including glycine in place of alanine in the hydrophilic positions weakened heparin binding, and peptides in which arginine was included in all basic positions displayed higher affinity for heparin than did those containing arginines and lysines. The latter is consistent with work showing a higher affinity interaction of arginine-heparin and arginine-HS than lysine-heparin or lysine-HS (36). This suggests that the heparin binding characteristics of the peptides developed here may rely on amino acid type and arrangement in addition to ionic interactions. Inclusion of prolines within or between consensus sequence motifs weakened affinity for heparin, possibly as a result of alterations in peptide secondary conformation; this issue was investigated in our CD experiments. Finally, changing the spacing between consensus motifs weakened affinity for heparin; however, sequence orientation did not appear to influence binding ability as long as the motifs were contiguous and in one orientation.

Molecular modeling of consensus sequences in native heparin-binding proteins predicts their presence within α-helical regions (6). Additionally, GAG-directed conformational changes on polypeptides such as poly-L-lysine and poly-L-arginine have been identified (26, 37, 38). Aqueous solutions of these polypeptides at neutral pH were shown by CD to adopt charged coil conformations and to display α-helical conformations in the presence of heparin. Our results showed that peptides of the type (AKKARA), have charged coil conformations at neutral pH. In the presence of heparin, however, a peptide that showed high affinity for heparin, (AKKARA)₆, underwent a conformational change to an α-helix. In the presence of excess heparin, a further conformational change produced a random coil structure. In contrast, a peptide that displayed weak heparin binding, (AKKARA)₁₀, failed to undergo any conformational change. Thus, the solution conformation of a peptide and its propensity to change conformation in the presence of heparin may be an indication of its ability to bind to heparin strongly. These data and those from experiments examining the effects of including prolines in peptides, which are known to disrupt the α-helical conformation, suggest that peptide secondary structure facilitates heparin binding.

Here we also examined the interaction between the high affinity heparin-binding peptide (ARKKAAKA), and EC PGs. Results showed that ECs secreted several types of PGs/GAGs that displayed significant affinities for (ARKKAAKA)₄ (K_d = 300 nM). ACE gel images revealed the resolution of multiple
PG/GAG species after their migration through the peptide-containing lanes, suggesting heterogeneity in PG/GAG charge, size, and/or binding affinities. It was found that the CS PGs or HS PGs likely bind the peptide similarly, because affinity was maintained even after treatment of total PGs with nitrous acid, which selectively degrades HS GAGs, heparinase I, or chondroitinase ABC. The free GAG chains had 3–4-fold lower affinity than the intact PGs. Thus, the core proteins of certain EC PGs may either contribute to binding directly or act as a tether to bring multiple GAGs into proximity for cooperative binding. Similar observations have been made previously for cartilage PG-type II collagen interactions (39) and SG-type I collagen interactions (40, 41). Our results are inconsistent with carbohydrate sequence selectivity in the binding of these peptides with EC PGs, because similar affinities for peptides were displayed by either total EC PGs or its CS PG fraction.

Of note is that the heparin-binding peptides designed here incorporate concatamers of heparin binding consensus sequences, which should rarely, if ever, appear in native proteins. Nonetheless, the proposed characteristics of heparin-binding motifs in proteins, as set forth by Cardin and Weintraub (6) based on their theoretical analysis of putative heparin-binding domains of native proteins, hold true with our model peptides. Thus, our data suggest that peptides containing the Cardin and Weintraub heparin-binding consensus sequences may show a selective advantage in heparin binding over certain other sequences that do not fit their criteria.

In summary, optimally active heparin-binding peptides should include multiple sequences of the types \((XBBXBX)_n\) and \((XBBXXBX)_n\). Sequence number and peptide \(M_n\) are the most critical features; peptides should be of at least approximately 30 residues, which could be decreased to 15 if cysteine is included near either terminus to promote dimerization. Peptides should contain contiguous sequence arrays, without intervening residues between sequences. Alanine, which stabilizes \(\alpha\)-helical conformation, should occupy the hydrophobic residue positions, and arginine should occupy the basic positions. The high affinity PG- or GAG-binding peptides developed here, or derivatives thereof, could prove useful as tools for the promotion of cell-substratum attachment of PG-expressing cells, in the targeting of drugs to PG-expressing cells and PG-rich extracellular matrices, or as antagonists of GAG-mediated actions, e.g. neutralization of the anticoagulant activity of heparin.

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