Characterization of Type XI Collagen-Glycosaminoglycan Interactions*

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Using competitive binding experiments, it was found that native type XI collagen binds heparin, heparan sulfate, and dermatan sulfate. However, interactions were not evident with hyaluronic acid, keratan sulfate, or chondroitin sulfate chains over the concentration range studied. Chondrocyte-matrix interactions were investigated using cell attachment to solid phase type XI collagen. Pretreatment of chondrocytes with either heparin or heparan sulfate significantly reduced attachment to type XI collagen. Incubation of denatured and cyanogen bromide-cleaved type XI collagen with radiolabeled heparin identified sites of interaction on the α1(XI) and α2(XII) chains. NH₂-terminal sequence data confirmed that the predominant heparin-binding peptide contained the sequence GKPGRGQRCPGPRGSGAR from the α1(XI) chain. Using rotary shadowing electron microscopy of native type XI collagen molecules and heparin-bovine serum albumin conjugate, an additional binding site was identified at one end of the triple helical region of the collagen molecule. This coincides with consensus heparin binding motifs present at the amino-terminal ends of both the α1(XI) and the α2(XII) chains. The contribution of glycosaminoglycan-type XI collagen interactions to cartilage matrix stabilization is discussed.

Type XI collagen is a component of the heterotypic collagen fibrillar network found in cartilage that, along with proteoglycan, gives cartilage its unique structural and biomechanical properties. The type XI collagen molecule consists of three genetically distinct polypeptide chains, namely α1(XI), α2(XII), and overglycosylated α1(II) chains, and is typical of the fibrillar class of collagens having a 300-nm triple helical domain (1). Although type XI collagen is a relatively minor collagen in cartilage, it is believed to be important in the regulation of fibril diameter (2) and in maintaining tissue integrity and cohesion. Mice homozygous for the autosomal recessive chondrodysplasia (cho) mutation in the coll11a1 gene (3) do not synthesize α1(XI) chains and have larger cartilage collagen fibrils, less cartilage matrix cohesion, and increased extractability of proteoglycans. However, neither the domains of type XI collagen responsible for these activities nor the molecules with which they interact are known.

By immuno-electron microscopy, it was found that the triple helical domain of type XI collagen was buried within the heterotypic fibril (4). However, it has been detected without the use of chaotropic agents both pericellularly (5) and, also, more generally throughout the matrix (4, 6), suggesting that some type XI collagen molecules in cartilage are not buried and are therefore available for interaction.

Type XI collagen has been shown to be associated with the surface of bovine articular chondrocytes in suspension culture (7). It is also known that the triple helical domain binds to heparin-agarose with greater affinity than other cartilage collagens, an activity that has been exploited as a tool for its purification (8). Therefore, the triple helix of type XI collagen may have the potential to bind glycosaminoglycans present on the surface of chondrocytes.

Although type XI collagen is associated with type II collagen in cartilage matrices, several studies show that chains of type XI and type V collagen can coexist within tissues (9–11). α1(XI) mRNA has also been detected in tumors (12), placenta-derived cell lines (13), and in a variety of noncartilaginous embryonic chick tissues (14). As a result, type V and type XI collagen chains are believed to participate in the formation of heterotypic molecules with stoichiometries not previously assigned. They are therefore considered as a single collagen type (15).

Much work has been done on the glycosaminoglycan and cell binding properties of type V collagen (for review, see Ref 15), and a region within the α1(V) chain has been identified as the site through which ionic interactions with glycosaminoglycans moieties occur (16). This site contains several basic amino acid residues and is located ~100 nm from the amino-terminal end of the triple helical domain of the molecule. Further studies (17) demonstrate that binding of heparin to residues 905–921 in this α1(V) site is influenced by secondary structure. The α1(XI) chain shows considerable homology with the α1(V) chain of type V collagen (12), and the sequence of amino acids comprising the heparin binding site within α1(V) is also present within α1(XI).

The recognition of heparin and heparan sulfates as surface components in various cell types (18, 19) suggests that type XI collagen may be important in cell-matrix interactions. The glycosaminoglycans associated with the cartilage matrix, however, are hyaluronic acid, chondroitin 4-sulfate, chondroitin 6-sulfate, dermatan sulfate, and keratan sulfate. In this study, we have used competitive binding experiments to determine the relative affinities of the type XI collagen molecule for various glycosaminoglycans. The contribution of heparinase- and collagenase-labile interactions to chondrocyte binding to type XI collagen has also been investigated. Furthermore, the use of rotary shadowing techniques has enabled localization of heparin binding sites on the triple helical molecule. These studies provide evidence for a more complex range of interactions be-

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 tween the triple helical domain of type XI collagen and glycosaminoglycans than was previously thought. Such interactions may be partly responsible for the role of type XI collagen in establishing and maintaining cartilage matrix integrity.

**EXPERIMENTAL PROCEDURES**

**Purification of Type XI Collagen and Preparation of Cyanojen Bromide Peptides**—Type XI collagen was isolated from porcine articular cartilage by limited pepdin digestion. The cartilage was extracted with 4 M guanidinium chloride in 0.05 M Tris-HCl buffer, pH 7.5, to remove proteoglycan. Pepsin (1 mg/100 mg of tissue) was dissolved in 0.5 M acetic acid and added to the insoluble material. After digestion at 4 °C overnight, insoluble material was sedimented by centrifugation, and the supernatant containing the pepsin-extractable material was precipitated sequentially with 0.7, 0.9, 1.2, and 2.0 M NaCl. The precipitates were collected by centrifugation. The 1.2 M precipitate fraction was redissolved in acid and reprecipitated. This was repeated until the fraction appeared to be homogeneous and free of other collagen types, as determined by SDS-polyacrylamide gel electrophoresis (PAGE)(20).

Cyanojen bromide (CNBs) peptides of type XI collagen were prepared by incubating 5 mg of purified pepsinized type XI collagen with 10 mM diithothreitol in phosphate-buffered saline (PBS) for 30 min at room temperature and subsequently with 5 mg of CNBs in 70% (v/v) formic acid. After digestion, the reaction mixture was dialyzed against 10-fold water with dialyzed extensively against 10 mM acetic acid, and lyophilized.

**Heparin Binding Studies**—To quantify binding of heparin to type XI collagen, native pepsinized type XI collagen diluted in 5 mM acetic acid was separated into aliquots and applied to nitrocellulose discs (4 mm in diameter. The coated discs were air-dried and incubated for 2 h in 96-well plates (Falcon) in 100 µl of PBS-Tween containing radiolabeled (N-[35S]sulfonate) heparin solution (15.8 mCi/g; 2 mCi/ml; 4–6-kDa molecule mass) (Amersham Pharmacia Biotech) at a final activity of 0.1–2.0 µCi/ml. After extensive washing in PBS-Tween supplemented with NaCl to a final concentration of 0.25 M, the amount of radiolabeled heparin bound to the discs was determined by scintillation counting.

To determine the specificity of the type XI collagen-glycosaminoglycan interaction, competitive binding assays were performed. Type XI collagen-coated nitrocellulose discs were incubated simultaneously with radiolabeled heparin (1 µCi/ml; 64 µg/ml) and unlabeled heparin, heparan sulfate, de-N-sulfated heparin, chondroitin 4-sulfate, chondroitin 6-sulfate, keratan sulfate, dermatan sulfate, and hyaluronic acid (0–1.05 mg/ml) in PBS. Unlabeled heparin preparations (from porcine intestinal mucosa) and other glycosaminoglycans were obtained from Sigma.

**Preparation of Chondrocytes**—Articular chondrocytes were isolated from porcine femoral head and condylar cartilage. The cartilage was digested with 0.4% (w/v) Pronase (protease E, Sigma) in Dulbecco’s modified Eagle’s medium containing 4 mM glutamine, 100 units/ml penicillin, 100 µg/ml streptomycin, 2.5 µg/ml amphotericin B, and 1% (v/v) fetal calf serum for up to 48 h.

**Cell Attachment and Blocking Assays**—Microwell plate wells were coated overnight with type XI collagen at 200 ng/well in 10 mM acetic acid at 4 °C. The plates were subsequently blocked at room temperature using a 1 mg/ml solution of bovine serum albumin in PBS. Control incubations were prepared by coating wells with 200 ng/well BSA. The plates were washed with PBS and used directly for the attachment assays.

Cells were harvested from the suspension cultures and incubated for 2 h with a highly purified bacterial collagenase preparation that exhibit no other proteinase activity (Form III, Advance Biofactures Corp.) at 1.5 units/ml or with heparin (Sigma) at 10 µg/ml. The chondrocytes were centrifuged by centrifugation at 100 × g for 10 min, washed twice with serum-free Dulbecco’s modified Eagle’s medium by resuspension and centrifugation, and dispensed onto the washed plates at a concentration of 5 × 10⁴ cells/ml. Cells were allowed to attach for 90 min at 37 °C in a humidified atmosphere containing 5% (v/v) CO₂. The plates were rinsed three times with PBS to remove unattached cells, and the number of attached cells was determined by measuring the N-acetylhexosaminidase activity (21). For subsequent experiments, collagenase-pretreated cells were washed and incubated on coated plates as above in serum-free Dulbecco’s modified Eagle’s medium in the presence or absence of either heparinase (heparinase III from Flavobacterium hepari- numa, Sigma) at 2.5 units/ml or heparin at 10 units/ml.

**Identification of Binding Sites on Whole Chains and CNBr Peptides of Type XI Collagen**—Whole and cyanogen bromide digests of purified pepsinized type XI collagen were resolved using SDS-polyacrylamide gel electrophoresis and electroblotted onto polyvinylidene difluoride (PVDF) membranes (Immobilon-P, Amersham Pharmacia Biotech) (22). After incubation in PBS containing 0.05% (v/v) Tween 20, the membranes were incubated with 35S-labeled heparin at 2 µCi/ml for 2 h at room temperature. After washing with PBS-Tween containing 0.25 M NaCl for 2 h, the blots were air-dried and exposed to Hyperfilm-MP radioimaging film (Amersham Pharmacia Biotech) for 5 days.

To isolate CNBr peptides containing heparin binding sites, CNBr digests (5 mg) of type XI collagen in 10 mM sodium phosphate buffer, pH 7.4, containing 0.15 M NaCl were incubated overnight at 4 °C with an aliquot of heparin-Sepharose (Amersham Pharmacia Biotech) equilibrated in the same buffer. After extensive washing of the heparin-Sepharose with 0.25 M NaCl in the same buffer, the adsorbed peptide(s) was eluted using 0.5 M NaCl, incubated with SDS-PAGE sample buffer at 60 °C for 30 min, and resolved on a 10% (w/v) polyacrylamide gel.

**Amino Acid Sequence Analysis**—Peptides separated by SDS-PAGE on a 10% (w/v) polyacrylamide gel were electroblotted onto nitrocellulose membrane (Applied Biosystems) in 10 mM CAPS buffer, pH 11. NH₂-terminal sequences were determined by automated Edman degradation on a protein sequencing system (Applied Biosystems) at the University of Manchester.

**Identification of Binding Sites by Rotary Shadowing Electron Microscopy**—Whole Type XI Collagen and Heparin-BSA Conjugate—Type XI collagen was solubilized in 10 mM acetic acid at a concentration of 1 mg/ml. An aliquot of the collagen (25 µl) was added to 1.25 ml of 20 mM ammonium bicarbonate, pH 8, containing 25 µl of commercially available BSA-conjugated heparin (Sigma). The mixtures were incubated at 4 °C for 24 h, and subsequently, glycerol was added to the mixture to give a final concentration of 30% (v/v). Samples were prepared by the sandwich method. 25 µl of sample was spread between two 1.5-cm² sheets of freshly cleaved mica (Agar Scientific Ltd., UK), which were separated after 1 min and dried under vacuum in a metal-coating system (Edwards 306). The samples were rotary-shadowed with platinum at an angle of 8° and subsequently coated with pulse-evaporated carbon at 90°. The replicates were floated onto distilled water, picked up on 400-mesh copper grids, air-dried, and examined by electron microscopy (Philips EM 400 or EM 208). Images were recorded at 50,000× magnification and scanned at 600 dots/inch using an Epson GT-7000 scanner. Measurements of molecular length and respective sites of heparin-BSA binding were acquired using a Windows 95 ProScan image analysis program after calibration with reference to the 8.75 crystal lattice-spacing of beef liver catalase.

**RESULTS**

**Binding of Heparin to Native Type XI Collagen**—Heparinagarose has been employed successfully for the affinity purification of type XI collagen, both before and after pepsinization. To ascertain the specificity of this binding with respect to glycosaminoglycan species, a nitrocellulose membrane solid phase assay was established in which heparin-collagen binding could be optimized and quantified (Fig. 1). Optimal concentrations of the collagen and of the radiolabel were established to be 10 µg of type XI collagen/disc and 1 µCi/ml (64 µg/ml) radiola beled heparin. The heparin was maintained at ∼64 µg/ml for subsequent assays.

The stoichiometry of the binding could not be calculated on a molar or weight ratio by introducing unlabeled heparin preparations of a range of molecular weight (average) into the assay (Fig. 2). The relationship between the molecular weight of the glycosaminoglycan species and inhibition of radiolabel bound was not linear. On a molar basis, this nonlinearity was accentuated further with the concentration of the 3-kDa (average) heparin preparation, required to reduce bound radiolabel by
The specificity of heparin binding site(s) was investigated by introducing unlabeled glycosaminoglycan species into the assay. Relative specificity of the glycosaminoglycans was ascertained by their capacity to compete with the radiolabeled heparin for type XI collagen binding. Over the concentration range studied, there appeared to be a hierarchy of binding affinity of the glycosaminoglycans (Fig. 3). None of the glycosaminoglycans could compete with radiolabeled heparin as successfully as heparin itself. Both heparan sulfate and dermatan sulfate significantly inhibited binding of radiolabeled heparin in a concentration-dependent manner. Hyaluronic acid, chondroitin 4-sulfate, chondroitin 6-sulfate, and keratan sulfate were all ineffective over the concentration range studied (up to 1.05 mg/ml) (Table I).

Attachment of Chondrocytes to Native Type XI Collagen—To address the possibility that glycosaminoglycan type XI collagen interactions may be of importance at the chondrocyte-matrix interface, the binding of chondrocytes to native type XI collagen was investigated using a cell binding assay. Fig. 4a summarizes the observed effects of chondrocyte pretreatment with exogenous heparin and bacterial collagenase on attachment to type XI collagen. The highly purified bacterial collagenase preparation used only digests regions containing the Gly-X-Y repeat of collagen, without digesting the noncollagenous proteins or domains. Pretreatment of cells with purified bacterial collagenase to remove cell surface collagen reduced the binding. Heparin also significantly reduced cell attachment to type XI collagen (p < 0.05) but also increased nonspecific binding to BSA, suggesting that specific binding was reduced to a greater extent. This indicates that heparin inhibits chondrocyte interaction with type XI collagen.

To ascertain whether the inhibitory effect of exogenous heparin was due directly to a cell surface heparin-like molecule, chondrocytes pretreated with collagenase were incubated with heparinase (Fig. 4b). Heparinase and heparin treatment both significantly (p < 0.05) reduced the specific cell attachment, indicating that the type XI collagen-chondrocyte interaction observed in vitro was due in part to a cell surface heparan sulfate-like molecule.

Identification of Heparin Binding Sites in Denatured Type XI Collagen by Blotting and NH₂-terminal Sequencing—Type XI collagen binds to heparin-Sepharose with approximately the same affinity, whether it has been digested with pepsin or not.
The percentage of radiolabeled heparin bound to type XI collagen in the presence of unlabeled glycosaminoglycans at 1.048, 0.262, and 0.065 mg/ml is shown below. Binding of heparin in the absence of unlabeled glycosaminoglycan is taken as 100%. Each value represents the mean of duplicates.

<table>
<thead>
<tr>
<th>Glycosaminoglycan</th>
<th>1.048 mg/ml</th>
<th>0.262 mg/ml</th>
<th>0.065 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparin</td>
<td>2.5</td>
<td>4.1</td>
<td>6.9</td>
</tr>
<tr>
<td>Heparan sulfate</td>
<td>4.3</td>
<td>8.8</td>
<td>34.7</td>
</tr>
<tr>
<td>Dermatan sulfate</td>
<td>4.3</td>
<td>35.0</td>
<td>61.0</td>
</tr>
<tr>
<td>Chondroitin-6-sulfate</td>
<td>73.0</td>
<td>73.0</td>
<td>106.0</td>
</tr>
<tr>
<td>Chondroitin 4-sulfate</td>
<td>101.0</td>
<td>86.0</td>
<td>92.0</td>
</tr>
<tr>
<td>Hyaluronic acid</td>
<td>84.0</td>
<td>85.0</td>
<td>96.0</td>
</tr>
<tr>
<td>Keratan sulfate</td>
<td>110.0</td>
<td>111.0</td>
<td>113.0</td>
</tr>
</tbody>
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FIG. 4. Chondrocyte attachment to type XI collagen. a, chondrocytes were harvested from suspension cultures and incubated for 2 h with purified bacterial collagenase or heparin. After washing, the cells were plated on microtiter plate wells coated with type XI collagen (solid bars) or BSA (hatched bars). b, chondrocytes pretreated with collagenase were incubated in type XI collagen or BSA-coated microtiter plate wells in the presence of heparin or heparinase. The values represent the absorbance of the product of the colorimetric assay used to quantify numbers of cells attached to the wells. The values shown are means ± S.E. of mean (n = 6).

The individual chains was sufficient for heparin binding. Heparin did not bind to the α1(II) chain.

The binding of heparin to electroblotted CNBr peptides of type XI collagen indicated that the binding site was not susceptible to CNBr cleavage (Fig. 5, lanes 3 and 4). NaCl concentrations of greater than 0.35 M were required to elute the radiolabel from the electroblotted type XI collagen chains or peptides (data not shown), suggesting that the affinity of binding to denatured collagen was similar to that of native type XI collagen.

CNBr peptides containing a heparin binding site were adsorbed onto a heparin-Sepharose column at a NaCl concentration of 0.25 M and, following extensive washing of the column with the same buffer, were eluted using a NaCl gradient, a peak of eluant occurring at around 0.375 M NaCl (data not shown). This experiment was repeated using batch-wise elution of heparin-Sepharose to which type XI collagen CNBr peptides had been bound (Fig. 6). CNBr peptides of type XI collagen were applied to heparin-Sepharose at 0.15 M NaCl. After washing in 0.25 M NaCl, the heparin-Sepharose was washed with an equal volume of 0.5 M NaCl. The peptides contained in each fraction are shown in Fig. 6. The eluted peptides were resolved on a 10% (w/v) polyacrylamide gel and electrotransferred onto polyvinylidene difluoride (Problott). Several faint bands are apparent in the 0.5 M NaCl fraction. However, one main band was seen, as described earlier. NH2-terminal sequencing revealed that the peptide was the product of CNBr cleavage of α1(XI) at methionine 792 and commenced with the sequence GLKGDRGEVGQ. From the human α1(XI) sequence (12) it was deduced that this peptide would contain the sequence identified as the heparin binding site within α1(V), GKFPGPRGIGPTGPRGSRGARGP (16), confirming that this homologous region is a binding site in both type XI and V collagen. An equivalent α2(XI) CNBr peptide bound to heparin-Sepharose was not isolated.

Rotary Shadowing of Native Type XI Collagen Triple Helix and Heparin-BSA Conjugate—To establish whether heparin binds to other sites along the triple helix, the binding of type XI collagen to a heparin-BSA conjugate was observed using rotary shadowing (Fig. 7a); the heparin conjugate had an approximate stoichiometry of five heparin molecules per BSA molecule (information from supplier). Large aggregates were often ob-
We have investigated the glycosaminoglycan binding properties of the 300-nm triple helical domain of type XI collagen to evaluate further its potential to interact with proteoglycan species in cartilage, thereby extending previous studies on the type XI collagen-glycosaminoglycan interaction (8, 23). Previously we have found that type XI collagen interacts with other matrix molecules in cartilage, necessitating the use of SDS to extract it from growth plate and articular cartilage (6) after pepsin digestion.

From the current study, heparin binding to type XI collagen appears to be independent of the triple helical conformation as it interacts with both native and denatured preparations of the collagen. The glycosaminoglycan specificity of the binding to the native triple helical molecule is very similar to that reported for other heparin binding molecules (24), with heparin and heparan sulfate showing the greatest affinity for the type XI collagen. As confirmation that the specificity observed was due to charge density alone, the nonphysiological but highly sulfated glycosaminoglycan, dextran sulfate, containing four sulfate groups per disaccharide and an average molecular mass of 10 kDa (Sigma), was found to bind with greater affinity than heparin itself (data not shown). Furthermore, in the current study, de-N-sulfated heparin did not interact with type XI collagen.

In our cell binding studies, we found that chondrocytes bind to type XI collagen and that this binding was reduced when cells were incubated with heparin or heparinase, suggesting that type XI collagen binds to a cell surface heparan sulfate proteoglycan. Previous studies (7) showed that chondrocytes in culture retained type XI collagen at the cell-medium interface, whereas type II collagen could be released into the medium. Also, immunolocalization studies showed that the triple helix of type XI collagen and, presumably, the heparin binding site are accessible in pericellular regions in cartilage (25, 5). It may be speculated, therefore, from the specificity of the binding, that type XI collagen triple helix binds to the small heparan sulfate or dermatan sulfate containing proteoglycans that are present in cartilage, particularly at the cell surface.

The binding of type XI collagen to cell surface proteoglycan may be important in the organization and stabilization of the cartilage matrix. Type XI collagen can regulate type II collagen metabolism (26), and as fibrillogenesis is initiated at the cell surface, the anchoring of type XI collagen may in some way control this process in vivo. Although alternative splicing of the NH2-terminal domains of type XI collagen (27, 28) indicates an important role for these domains in cartilage development, it is not known whether these proteolytically susceptible domains (29, 30) are retained in adult cartilage. Therefore, the interactions of proteoglycans with the triple helical domain may be important for maintaining tissue integrity, particularly in the pericellular environment.

We have identified two heparin binding sites on type XI collagen at around 24 and 95 nm from the NH2-terminal of pepsin-extracted collagen. The location of the 25-nm site is derived from the rotary shadowing data that indicate the presence of a site at around 280 nm from one end of the helix, which is located around 70 nm from the 95- or 100-nm site identified by sequence analysis and from previous studies. The 95-nm site identified by rotary shadowing coincides with the sequence obtained from the heparin-binding CNBr peptide of α1(XI) (Fig. 7a, lower left and lower middle panels). The frequency distribution of binding along the type XI collagen triple helix is presented in Fig. 7b.

FIG. 7. Rotary shadowing of heparin-BSA conjugate bound to pepsinized type XI collagen. Type XI collagen was incubated with heparin-BSA conjugate and visualized as described under "Experimental Procedures." a, two predominant binding sites were observed, at ~95 nm (upper six panels) and at 280 nm (lower left and center panels). Occasional molecules exhibited binding of heparin-BSA at both sites simultaneously (lower right panel). Bar, 50 nm. The distribution data for a representative number of labeled molecules are summarized in the histogram (b).
8a). Both the α1(XI) and α2(XI) chains of type XI collagen contain, in register, the motif of basic residues identified in the α1(V) chain as the 100-nm heparin binding site. It is surprising therefore, that the corresponding α2(XI) CNBr peptide was not identified by binding of radiolabeled heparin. The sequence of amino acids in α2(XI) is not identical to the KPGPRGQR sequence in α1(V) and α1(XI), defined as the heparin binding motif (31). This difference would not, however, explain the anomaly that the whole α2(XI) chain can bind heparin, whereas the CNBr peptide containing the postulated site does not.

A recent study of type V collagen heparin binding has indicated that the stoichiomteries of type V collagen chains present may contribute to binding sites of varying affinities (17) due to the presentation of basic residues on one face of the polyproline II helix of each α1(V) chain. Thus, the α1(V)2 homotrimer has a greater affinity than the α1(V)2α2(V) heterotrimer. We have not been able to isolate the α2(V) peptide that corresponds to the 100-nm site or that can bind heparin within the denatured α2(XI) chain. Nevertheless, the α2(XI) chain may contribute to the 100-nm site in the α1(XI)2α2(XI) or α1(XI)2II heterotrimer, akin to the α1(V)2α2(V) molecule. Similar to the α1(V)2α2(V) heterotrimer, this site in type XI collagen would have positive charges on the outside of the two contributing α-chains and would not form a continuous band around the triple helical molecule. The 20-amino acid sequences depicted in Fig. 8a represent a distance of ~6 nm in the triple helix, which would accommodate 7 disaccharide units. If the complete binding site is required for optimal binding, this may explain why the 3-kDa heparin (average 6 disaccharides) did not bind as well as the larger heparin molecules. However, if the sequence (KPGPRGQR) is correct, the heparin binding motif (31) at this site, where X represents any amino acid, while B represents a basic amino acid. Such a site has been implicated in binding the triple helical collagen tails of asymmetric acetylcholinesterase to heparin (33). However, sequences R581 to R588 and R539 to R546 in the α1(XI) and α2(XI) chains respectively, also agree with the heparin binding motif described earlier which accommodates a pentasaccharide (31). Thus, the α1(XI), α2(XI) and α1(V) chains have potential to bind heparin at the NH2-terminal end by either of these motifs. While we have identified this site in type XI collagen by rotary shadowing, this site has not been reported for type V collagen.

The corresponding type XI collagen CNBr peptides of ~5 kDa were not detected in the assay in which radiolabeled heparin was bound to CNBr peptides electrophlotted onto nitro-cellulose. In both the α1(XI) and α2(XI) chains however, the site is followed immediately by a methionine that would be cleaved by CNBr. This may prevent the CNBr peptide assuming a structure suitable for heparin binding. It has been suggested that there is a requirement for conformational constraint within a polyproline II helix on the heparin binding sequence of each chain; flanking sequences would influence this secondary structure (17). The sequences may however also need the constraints of being in a triple helical structure to function as an efficient binding site.

It is unlikely that multivalent binding can occur between a single glycosaminoglycan chain and both sites identified on one type XI collagen molecule, either in our in vitro studies or in vivo, due to spatial constraints. However, it is possible that both sites are accommodated by glycosaminoglycan chains present on different cell surface heparan sulfate proteoglycans. This, along with the formation of cross-links between type XI collagen monomers (34), presents the possibility that type XI collagen-glycosaminoglycan interactions contribute to cell surface-matrix interactions of a range of complexity and affinity. Indeed, the sum of a range of interactions between type XI collagen and matrix or cell-associated proteoglycans may be advantageous to matrix stabilization during periods of dynamic remodeling. The interactions between the triple helical domain of type XI collagen and glycosaminoglycan species may contribute therefore, to the cohesiveness and integrity of articular cartilage matrix.

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