Interactions of syndecan-1 and heparin with human collagens

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Introduction

Heparan sulphate—collagen interactions are thought to play important roles in cell adhesion and in the formation of extracellular matrix structures such as basement membranes (Stamatoglou and Keller, 1982; Koda et al., 1985; Piepork and Chapman, 1985; Tsilibary et al., 1988; Koliakos et al., 1989; Sanderson et al., 1992). The biochemistry of the interactions between heparin/heparan sulphates and the collagens is poorly understood, and previous work on this topic has produced conflicting results. For example, some workers have reported that heparin and heparan sulphate proteoglycans (HSPGs) bind to type I collagen (Koda et al., 1985; Stamatoglou and Keller, 1982; Keller et al., 1986; San Antonio et al., 1992), but others disagree (Yaoi et al., 1990). Similarly, some have reported binding between heparin-like molecules and type II collagen (Keller et al., 1986), while others observed no interaction between these molecules (Koda et al., 1985; LeBaron et al., 1989; Yaoi et al., 1990). Finally, there are reports that heparin or HSPGs bind to type IV collagen (Tsilibary et al., 1988; Koliakos et al., 1989), as well as reports that these molecules do not bind type IV collagen (Koda et al., 1985; LeBaron et al., 1989; Yaoi et al., 1990). These contradictory conclusions may reflect differences in the tissue sources or conditions used for the isolation of the collagens, glycosaminoglycans (GAGs) or HSPGs, differences in the techniques used for binding assays, or differences in the interpretation of results from qualitative or semi-quantitative assays.

To better understand the nature of the interactions between HSPGs or heparin and the collagens, we have examined these interactions using affinity co-electrophoresis (ACE) (Lee, M.K. and Lander, A.D., Proc. Natl. Acad. Sci. USA, 88, 2768–2772, 1991) to study the binding of the heparan sulphonate proteoglycan syndecan-1 and heparin to human collagens. [135]Syndecan-1 from normal murine mammary gland (NMuMG) epithelial cells and low-M₆ (~6 kDa) [125]heparin were subjected to electrophoresis through agarose gel lanes containing human collagens at various concentrations, and binding affinities were measured from shifts in migration of the labelled materials. Results demonstrate that the affinities of each collagen for syndecan-1 and low-M₆ heparin were similar, and followed the order: type V > type IV = type III > type I > type VI > type II, and ranged in Kₐ from ~10⁻⁸ to ~3 × 10⁻⁶ M. These data suggest that syndecan-1 and heparin may contain similar collagen-binding determinants. It was also found that the same heparin subpopulation was selectively bound with high affinity by each of the collagens. The published amino acid sequences of the six collagens were examined for what are thought to be heparin-binding consensus sequences (Cardin, A.D. and Weintraub, H.J.R., Arteriosclerosis, 9, 21–32, 1989). The presence of such sequences did not correlate with affinity for heparin or syndecan-1, and collagens I, II and III lacked such sequences entirely. The data suggest that collagens may use novel types of binding sites to interact with GAGs.

Key words: cell adhesion/extracellular matrix/glycosaminoglycans/heparan sulphate proteoglycans

Results

[135]Syndecan-1 and low-M₆, [125]Tyramine-heparin ([125]Tyr-heparin) were subjected to electrophoresis through agarose gel lanes containing various concentrations of each of six types of human collagen. The resulting electrophoretograms (Figure 1) revealed that the mobilities of both heparin and syndecan-1 were shifted by each collagen in a concentration-dependent manner. The collagen concentrations over which shifts in mobility occurred were different for each collagen type, but for any single collagen appeared grossly similar for heparin and syndecan-1. In contrast, no interaction was observed between [135]chondroitin sulphates and human collagens types I or II (tested at protein concentrations of up to 1 μM; data not shown).

Retardation coefficients (R) were measured for each lane of the electrophoretograms shown in Figure 1, and are plotted as...
Collagen Syndecan Heparin

Fig. 1. ACE analysis of the interactions between \( ^{1}^{2} \)S syndecan-1 or low-M, \( ^{1}^{2} \)H Tyr-heparin and various human collagens. ACE gels were constructed containing the human collagens indicated at the concentrations (nM) shown beneath each gel lane. Radiolabelled syndecan or heparin was loaded into a sample slot (located above each gel), and electrophoresis was conducted towards the anode (located below each gel). Images of syndecan-1 or heparin migration patterns within ACE gels were obtained using a phosphorimager (Molecular Dynamics). From these electrophoretograms, the dissociation constant \( K_{d} \) can be estimated from the protein concentration at which the syndecan or heparin is half-shifted from being fully mobile at very low protein concentrations or between protein-containing lanes, to being maximally retarded at high protein concentrations (Lee and Lander, 1991).

A function of collagen concentration in Figure 2. From these data, apparent \( K_{d} \)s for syndecan-1 and heparin binding to all six collagens were derived, and are presented in Table I. The results indicate that the affinities of the collagens for both syndecan-1 and low-M, heparin ranged in \( K_{d} \) from \( \sim 10 \) nM to \( \sim 3 \) \( \mu \)M. In general, syndecan-1 was bound by the collagens with somewhat higher apparent affinity than was heparin (Table I, ratios).

Further examination of the electrophoretograms in Figure 1 indicates that when the concentrations of each of the collagens were close to the \( K_{d} \) for heparin binding, low-M, heparin did not migrate as a sharp band of intermediate mobility, but rather as a broad smear that included material of both high and low mobility. Similar behaviour was described in detail for the binding of low-M, heparin to rat tail tendon type I collagen (as well as to laminin and fibronectin, but not thrombospondin or fibroblast growth factor) and could be shown to be caused by differences in collagen affinity for different subpopulations of heparin molecules (San Antonio et al., 1993).

To confirm that human collagens I–VI also display selectivity for heparin subpopulations, as well as to determine whether the heparin species selected for by each collagen type were similar or different, we used preparative ACE to isolate fractions of heparin that differ \( \sim 15 \)-fold in their affinity for rat tail tendon type I collagen. Briefly, low-M, heparin was subjected to electrophoresis through agarose containing non-pepsinized, rat tail tendon type I collagen at a concentration of 75 nM. A heparin fraction representing the 25% of starting material that was most strongly bound by type I collagen, as well as a fraction representing the 25% most weakly bound by type I collagen, were recovered from the gel (cf. San Antonio et al., 1993). These two heparin subpopulations were then tested for their binding to each of the human collagens by ACE (Figure 3). It was found that all of the human collagens showed tighter binding to the heparin fraction that had bound most tightly to rat type I collagen, than they had to unfractionated heparin. Overall, differences in affinity of 5- to 10-fold were observed when fractions that had bound strongly and weakly to rat type I collagen were compared for binding to human collagens (Table I, Figure 3).
Syndecan-1 and heparin interactions with collagens

A.

Heparin Fraction

|        | I      | III | V     |
|--------|--------|-----|-------|
| CL I-S | ![Image](image1.png) | ![Image](image2.png) | ![Image](image3.png) |
| CL I-W | ![Image](image4.png) | ![Image](image5.png) | ![Image](image6.png) |

[Collagen], nM

B.

Fig. 3. ACE analysis of the interactions between heparin subpopulations and human collagens. Heparin subpopulations that bind to type I collagen strongly or weakly were isolated by electrophoresis of \[^{[12]}I\]Tyr-heparin through agarose containing rat tendon type I collagen at a concentration of 75 nM. Heparin fractions containing the most retarded 25% of the heparin (designated ‘CL I-S’) and the least retarded 25% of the heparin (designated ‘CL I-W’) were recovered from the gels and subjected to electrophoresis against human collagens using ACE. (A) Electrophoretograms are from experiments in which CL I-S and CL I-W were analysed for their binding to collagen types I, III and V. Images of heparin migration patterns within ACE gels were obtained using a phosphorimager (Molecular Dynamics). (B) Calculation of affinities of heparin subpopulations for human collagens. Heparin retardation coefficients (R) within each protein-containing lane were determined (see Materials and methods, and the legend to Figure 2), and are plotted against protein concentration for collagen types III and V.

Discussion

We have used ACE to determine the apparent dissociation constants for the binding of the extracellular domain of syndecan-1 or of low-M\textsubscript{r} heparin by each of six human collagen types. In contrast to some previous observations on heparan sulphate/heparin–collagen interactions (see Introduction), we found that all collagen types studied, except for type II collagen, showed relatively high-affinity interactions with syndecan-1 and heparin. The affinities of the collagens for syndecan-1 and low-M\textsubscript{r} heparin followed the order: type V >> type IV = type III = type I > type VI >> type II, and ranged in \(K_d\) from \(\sim 10\) nM to \(\sim 3\) \(\mu\)M. The highest affinity interactions observed were those between type V collagen and syndecan-1 or heparin, and are comparable to affinities we have measured between basic fibroblast growth factor or thrombospondin and low-M\textsubscript{r} heparin (San Antonio \textit{et al.}, 1993). Collagen types I, III, IV and VI exhibited intermediate affinities for syndecan-1 and heparin, in the range that has previously been observed for the interactions of various other heparin-binding extracellular matrix proteins to syndecan-1 or to heparin (Sanderson \textit{et al.}, 1992; San Antonio \textit{et al.}, 1993). Finally, the interaction between type II collagen and syndecan-1 or heparin was detectable, but considerably weaker than that of the other collagens.

The fact that previous studies on this topic have produced such conflicting results is probably due to differences in the methods used to study heparin or HSPG–collagen interactions.
For example, many previous studies have examined the binding of GAGs or HSPGs to collagen samples that had been adsorbed to plastic surfaces. Because collagen—GAG interactions are dependent on maintaining collagen in its native conformation (Koda et al., 1985; Keller et al., 1986), it is possible that adsorption of collagens to plastic alters their conformation and thus their binding properties. Similarly, some workers have examined collagen—heparin interactions in the presence of high concentrations of urea (Yaoi et al., 1990), which may also disrupt conformation. Finally, most studies cited above also employed collagens or GAGs that had been covalently linked to insoluble matrices, or have been covalently modified to facilitate radioisotopic labelling—such manipulations can affect the binding properties of these molecules (cf. San Antonio et al., 1993). In the present study, the use of the ACE technique to analyse GAG—collagen binding eliminates many of these concerns because the proteins are likely to exist in their native or near native states within the agarose gel, and because the method for the tyramide end-labelling of low-M\(_r\) heparin used here probably does not significantly affect the protein-binding characteristics of the heparin chains (San Antonio et al., 1993).

Overall, it was found that syndecan-1 was bound with slightly higher affinities by the collagens than was heparin. This may occur because syndecan-1 contains several heparan sulphate chains (Rapraeger et al., 1985) and, unlike low-M\(_r\) heparin, is capable of multivalent binding interactions with the collagens. It is also possible, however, that the collagen-binding sites on syndecan-1 heparan sulphate are of intrinsically higher affinity than those on the average low-M\(_r\) heparin molecule. It may be noteworthy in this regard that a subpopulation of low-M\(_r\) heparin chains, isolated by virtue of strong binding to rat tendon type I collagen, was bound to each of the human collagens as strongly as syndecan-1. We have also shown that the isolated heparan sulphate chains of syndecan-1, obtained by protease and chondroitinase ABC treatment of normal murine mammary gland (NMuMG) syndecan-1, are bound by rat type I collagen with an affinity similar to that observed for the binding of intact syndecan-1 (Sanderson et al., 1994). Thus, under the conditions of our experiments, the core protein of syndecan-1 does not contribute to collagen binding.

It is interesting that the same subpopulation of low-M\(_r\) heparin that was previously shown to be preferentially bound not only by rat tendon type I collagen, but by laminin and fibronectin as well, also bound strongly to each of the human collagens (San Antonio et al., 1993). Previous studies indicate that the basis for the selective binding of rat type I collagen to distinct heparin subpopulations is not simply heterogeneity in GAG charge or M\(_r\), but involves heterogeneity in the distribution and abundance of structural features of heparin chains, such as specific sequences (Lee and Lander, 1991; San Antonio et al., 1993). Extending this argument, we suspect that the basis for the selective binding of the human collagens to heparin subpopulations depends on similar aspects of heparin structure. Another protein which is known to bind selectively to heparin subpopulations is antithrombin III (Jordan et al., 1979). It remains to be determined whether the selectivity displayed by the collagens for heparin subpopulations can be attributed to oligosaccharide sequences, as has been done for antithrombin III [see Marcum and Rosenberg (1989) for a review].

Just as specific structures on GAG chains are likely to mediate binding to collagens, so are specific sites on collagens likely to mediate binding to GAGs. Indeed, recent electron microscopic evidence suggests that heparin interacts with type I collagen at a single major (and possibly one minor) site within each tropocollagen monomer (San Antonio et al., 1994). Recently, it has been proposed that the heparin-binding sites on proteins can be recognized by the presence of either of two consensus amino acid sequences, each containing clusters of several basic and hydrophobic amino acids, thought to be present within \(\alpha\)-helical domains (Cardin and Weintraub, 1989). Such sites should be rare or non-existent in the pepsinized collagen preparations used in the present study, in which most or all of the non-triple helical domains are degraded. Thus, the fact that these collagens bound heparin with high affinity, and with similar affinities to those observed for the binding between non-pepsinized type I collagen and heparin (San Antonio et al., 1992, 1993), suggests that typical heparin-binding consensus sequences do not play a significant role in the binding of heparin by the collagens. Nonetheless, we have examined collagens I—VI for the presence of heparin-binding consensus sequences. We find that the chains of type I, II and III collagen lack such sequences (Kuivaniemi et al., 1988; Tromp et al., 1988; Janeczko and Ramirez, 1989; Su et al., 1989). Moreover, the \(\alpha\)1 and \(\alpha\)2 chains of type IV collagen (Soininen et al., 1987; Hostikka and Tryggvason, 1988), and the \(\alpha\)1 chain of type VI collagen, also lack these sites (Chu et al., 1989). On the other hand, the \(\alpha\)5 chain of type IV collagen, which is not present in the preparation of type IV collagen used here, contains two such heparin-binding consensus sequences (one in the triple helical domain and one in the non-collagenous domain) (Pihlajaniemi et al., 1990); the \(\alpha\)1 and \(\alpha\)2 chains of type V collagen contain four (only one is in a triple helical domain) (Myers et al., 1985; Takahara et al., 1991), and the \(\alpha\)2 and \(\alpha\)3 chains of type VI collagen contain five (none are in triple helical domains) (Chu et al., 1989, 1990).

From the above discussion, it is clear that not all of the collagens (i.e. types I and III) that bind heparin with high affinity contain what have been proposed to be heparin-binding amino acid sequences. The data imply that collagens use novel heparin-binding sites to interact with HSPGs or heparin. This conclusion is not surprising in view of the fact that the proposed heparin-binding consensus sequences are thought to require an \(\alpha\)-helical conformation (Cardin and Weintraub, 1989), and such a conformation should not occur commonly, if at all, within the largely triple helical collagens. The very strong affinity of type V collagen for syndecan-1 and heparin may be due to the fact that the constituent chains of type V collagens are, collectively, the most basic chains of all the collagens (Miller and Gay, 1987). Therefore, ionic interactions, coupled with a unique spatial arrangement of amino acid side chains in the native triple helical collagen molecules, may account for the preferential binding of syndecan-1 and heparin by native type V collagen.

Materials and methods

Materials

Human collagens were prepared as detailed elsewhere (Miller and Rhodes, 1982; Gay et al., 1988). In brief, collagen types I, III and V were from embryonic human skin, prepared by extraction with 0.5 N acetic acid containing 1 mg/ml pepsin. The collagen types were separated by repeated sequential salt precipitations, and were further purified by DEAE–cellulose and carboxymethyl cellulose chromatography under native conditions (Gay and Miller, 1979; Kresina and Miller, 1979). Collagen type II was from human fetal articular cartilage, isolated as described previously (Miller and Rhodes, 1982). Collagen type IV was from human amnion basement membrane, isolated under
similar conditions as those outlined for the isolation of types I, III and V collagen. Type V collagen was from pepsinized human placenta, purified by salt precipitation. Collagen purity was determined by SDS–PAGE analysis and amino acid analyses on acid-solubilized samples (Miller, 1972). Type I collagen from rat tail tendons was isolated as described previously (San Antonio et al., 1992). In brief, rat tail tendons were subjected to three cycles of extraction/purification with 0.5 N acetic acid/7.5% NaCl, and purity was assessed by SDS–PAGE. Collagen M₃₃ was taken to be: types I, II, III and V = 300 000; type IV = 900 000; type VI = 600 000.

Heparin (Grade I, porcine intestinal mucosa) was from Sigma. Heparin was substituted with tyrmine at the polysaccharide's reducing end (San Antonio et al., 1993), radiiodinated to a specific activity of ~30 000 Ci/mg and radiiodinated low-M₃₃ [¹²⁵I]Tyr-heparin chains of M₃₃ = 6000 were isolated by Sephadex G-100 chromatography (Lauren et al., 1978; Jordan et al., 1979; Rosenberg et al., 1979). The ectodomain of [³⁵S]syndecan-1 was prepared as detailed previously (Sanderson et al., 1992). In brief, syndecan-1 was metabolically labelled with [³⁵S]sulphate in cultures of NunMG epithelial cells. The extracellular domain of syndecan-1 was released from the cell monolayer by mild trypsinization, and was purified by DEAE chromatography, followed by affinity chromatography on Sepharose CL-AB matrix to which it was covalently linked an anti-syndecan core protein antibody, mAb281-2. Metabolically radiolabelled [³⁵S]chondroitin sulphates from day 8 chick lung bud mesenchyme cultures were the generous gift of Dr Michael J. Sorrell of Case Western Reserve University.

Methods

For ACE experiments, collagens were dissolved in 0.5 N acetic acid at concentrations ranging from 1 to 8 mg/ml, with gentle stirring overnight at 4°C. Collagens were then serially diluted in 0.5 N acetic acid. ACE was carried out as previously described (Lee and Lander, 1991). Briefly, a 1% low-melting-point agarose solution in 50 mM sodium 2-(N-morpholino)-hydroxypropyly sulphonic acid (MOPSO) (pH 7.0)/125 mM sodium acetate/0.5% CHAPS electrophoresis buffer was poured hot onto a piece of GelBond fitted within a plexiglass gel casting tray, in which a Teflon comb and strip were positioned. After the agarose solidified, the comb and strip were removed, leaving a 4 mm thick gel containing nine parallel 4 x 45 mm rectangular wells and a single 66 x 1 mm slot 2 mm away from the tops of each of the wells. To construct collagen-containing ACE gels, acid-solubilized collagen samples were rapidly neutralized by the addition of an equal volume of 0.5 N NaOH, and mixed with warm 2x concentrated electrophoresis running buffer and agarose minus the sodium acetate [1× running buffer was 50 mM sodium MOPSO/125 mM sodium acetate (pH 7.0)], and samples were poured into the agarose wells. Electrophoresis of radiolabelled GAGs or proteoglycans was then conducted as described previously (Lee and Lander, 1991). Briefly, a 1% low-melting-point agarose slab containing rat tail tendon type I collagen at 75 nM, to isolate syndecan-1 and heparin interactions with collagens

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Abbreviations

ACE, affinity co-electrophoresis; CL-J.S., heparin fraction strongly bound by type I collagen; CL-I.W., heparin fraction weakly bound by type I collagen; GAG, glycosaminoglycan; HSPG, heparan sulphate proteoglycan; MOPSO, 2-(N-morpholino)-hydroxypropyly sulphonic acid; NuMG, normal mouse mammary gland epithelial cell line. [¹²⁵I]Tyramine-labelled heparin.
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