UC Irvine

UC Irvine Previously Published Works

Title

Interactions of syndecan-1 and heparin with human collagens.

Permalink

<https://escholarship.org/uc/item/1fz0b98p>

Journal

Glycobiology, 4(3)

ISSN

0959-6658

Authors

San Antonio, JD
Karnovsky, MJ
Gay, S
et al.

Publication Date

1994-06-01

DOI

10.1093/glycob/4.3.327

Copyright Information

This work is made available under the terms of a Creative Commons Attribution License, available at <https://creativecommons.org/licenses/by/4.0/>

Peer reviewed

Interactions of syndecan-1 and heparin with human collagens

James D. San Antonio^{1,5}, Morris J. Karnovsky¹,
Steffen Gay², Ralph D. Sanderson³ and Arthur D. Lander⁴

¹Department of Pathology, Building D2, Room 440, Harvard Medical School, 200 Longwood Avenue, Boston, MA 02115, ²Department of Medicine, University of Alabama, Birmingham, AL, ³Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, AR and ⁴Department of Brain and Cognitive Sciences, and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA, USA

⁵To whom correspondence should be addressed

Glycosaminoglycan (GAG)–collagen interactions play important roles in cell adhesion and extracellular matrix assembly; however, the chemical bases for these interactions are not fully understood. We have used 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 sulphate proteoglycan syndecan-1 and heparin to human collagens. [³⁵S]Syndecan-1 [from normal murine mammary gland (NMuMG) epithelial cells] and low-*M_r* (~6 kDa) [¹²⁵I]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-*M_r* heparin were similar, and followed the order: type V >> type IV ≈ type III ≈ type I > type VI >> type II, and ranged in *K_d* 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/heparin binding site/heparan sulphate proteoglycans

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; Piepkorn 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 and Lander, 1991). With the ACE technique, binding is measured under non-denaturing conditions and at physiological ionic strength to intact proteins incorporated within an agarose gel. In addition, in the present study, techniques were used for the radiolabelling of syndecan-1 or of heparin chains that are unlikely to alter their protein-binding characteristics (San Antonio *et al.*, 1993). Using ACE, we have measured apparent dissociation constants for the interaction of the extracellular domain of syndecan-1, or of heparin, with each of six human collagen types. In addition, we have established that the human collagens selectively bind heparin subpopulations, as do certain other heparin-binding proteins, including laminin, fibronectin and rat type I collagen (San Antonio *et al.*, 1993). Finally, we have searched the published amino acid sequences of human collagens I–VI to determine if the presence of what are thought to be heparin-binding consensus sequences in each collagen type correlates with heparin-binding characteristics.

Results

[³⁵S]Syndecan-1 and low-*M_r* [¹²⁵I]Tyramine-heparin ([¹²⁵I]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 [³⁵S]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

Fig. 1. ACE analysis of the interactions between [^{35}S]syndecan-1 or low- M_r [^{125}I]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_r heparin ranged in K_d from ~ 10 nM to ~ 3 μM . 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_r 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_r 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 ~ 15 -fold in their affinity for rat tail tendon type I collagen. Briefly, low- M_r 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

Fig. 2. Calculation of affinities of syndecan-1 or low- M_r heparin for various human collagens. From each electrophoretogram in Figure 1, retardation coefficients (R) for syndecan-1 and heparin were determined (see Materials and methods), and are plotted against protein concentration. Smooth curves represent non-linear least-squares fits to the equation $R = R_\infty / (1 + (K_d/[\text{protein}])^2)$.

Table I. Apparent affinities of heparin, heparin subpopulations and syndecan-1 for human collagens. K_d s are derived from electrophoretograms such as those shown in Figures 1 and 3. Average K_d s represent data from two experiments using the same collagen preparations, except for data of heparin binding to type IV collagen (asterisk) which were obtained from three experiments using three different collagen preparations. Heparin fractions containing the most retarded 25% of the heparin (CL I-S) and the least retarded 25% of the heparin (CL I-W) were recovered from rat tail tendon type I collagen agarose gels as described in Results

CL	K_d (nM)			K_d (nM)		
	Heparin	Syndecan	Ratio	CL I-S	CL I-W	Ratio
I	315 $\pm$ 83	164 $\pm$ 7	1.9	92 $\pm$ 23	878 $\pm$ 94	9.4
II	1576 $\pm$ 617	2854 $\pm$ 1964	0.55	1152		
III	255 $\pm$ 52	181 $\pm$ 52	1.4	48 $\pm$ 2	264 $\pm$ 19	5.5
IV	239 $\pm$ 113*	124 $\pm$ 9	1.9	43		
V	16.6 $\pm$ 3	5.1 $\pm$ 6.1	3.3	4.8 $\pm$ 0.4	48 $\pm$ 6	10
VI	519 $\pm$ 64	321 $\pm$ 55	1.6	162		

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).

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 [^{125} 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_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_r heparin followed the order: type V $\gg$ type IV $\approx$ type III $\approx$ type I $>$ type VI $\gg$ type II, and ranged in K_d from ~ 10 nM to ~ 3 μ 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_r heparin (San Antonio *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 *et al.*, 1992; San Antonio *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 collagen 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 tyramine 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 α -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 α -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 VI was from pepsinized human placenta, also purified by salt precipitation. Collagen purity was determined by SDS-PAGE analysis and amino acid analyses on acidic hydrolysates (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/precipitation with 0.5 N acetic acid/7.5% NaCl, and purity was assessed by SDS-PAGE. Collagen monomer M_s were 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 tyramine at the polysaccharide's reducing end (San Antonio *et al.*, 1993), radioiodinated to a specific activity of ~30 000 c.p.m./ng and radioiodinated low- M_r [^{125} I]Tyr-heparin chains of $M_r \geq 6000$ were isolated by Sephadex G-100 chromatography (Laurent *et al.*, 1978; Jordan *et al.*, 1979; Rosenberg *et al.*, 1979). The ectodomain of [^{35} S]syndecan-1 was prepared as detailed previously (Sanderson *et al.*, 1992). In brief, syndecan-1 was metabolically labelled with [^{35} S]sulphate in cultures of NMuMG 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-4B matrix to which was covalently linked an anti-syndecan core protein antibody, mAb281-2. Metabolically radiolabelled [^{35} S]chondroitin sulphates from day 8 chick limb 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)-hydroxypropane 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 × 45 mm rectangular wells and a single 66 × 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 2× 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). Syndecan-1 or heparin mobility within ACE gels was measured using a phosphorimager (Molecular Dynamics) by scanning each protein lane and determining relative radioactivity content per 88 µm pixel along the length of the lane, resulting in a heparin distribution curve for each gel lane. Heparin mobility in each lane was taken as the pixel position that subdivided these curves into halves of equal area. Calculation of retardation coefficients (R), curve fitting of binding isotherms and determination of apparent K_d s were as described previously (Lee and Lander, 1991; San Antonio *et al.*, 1993).

For the preparative electrophoretic fractionation of heparin, in some experiments [^{125} I]Tyr-heparin was subjected to electrophoresis through a single agarose slab containing rat tail tendon type I collagen at 75 nM, to isolate heparin subpopulations that bind strongly and those that bind weakly to type I collagen (San Antonio *et al.*, 1993).

Acknowledgements

We gratefully acknowledge the gift of [^{35} S]chondroitin sulphates from Dr Michael Sorrell, Department of Biology, Case Western Reserve University, Cleveland, and the assistance of Dr Suk P. Oh and Dr Ernst Reichenberger of the Cell Biology Department of Harvard Medical School, Boston, in obtaining collagen sequence information. This work was supported by NIH NS 26862 to A.D.L., NIH HL 17747 to M.J.K., NIH CA 55879 to R.D.S., NIH DE 08520 to S.G. and an Eppeley Foundation Grant to J.S.A.

Abbreviations

ACE, affinity co-electrophoresis; CL I-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)-hydroxypropane sulphonic acid; NMuMG, normal mouse mammary gland epithelial cell line; [^{125} I]Tyr-heparin, [^{125} I]Tyramine-labelled heparin.

References

- Cardin, A.D. and Weintraub, H.J.R. (1989) Molecular modeling of protein-glycosaminoglycan interactions. *Arteriosclerosis*, **9**, 21–32.
- Chu, M.L., Pan, T., Conway, D., Kuo, H., Glanville, R.W., Timpl, R., Mann, K. and Deutzmann, R. (1989) Sequence analysis of $\alpha 1(VI)$ and $\alpha 2(VI)$ chains of human type VI collagen reveals internal triplication of globular domains similar to the A domains of von Willebrand factor and two $\alpha 2(VI)$ chain variants that differ in the carboxy terminus. *EMBO J.*, **8**, 1939–1946.
- Chu, M.L., Zhang, R.Z., Pan, T., Stokes, D., Conway, D., Kuo, H., Glanville, R., Mayer, U., Mann, K., Deutzmann, R. and Timpl, R. (1990) Mosaic structure of globular domains in the human type VI collagen $\alpha 3$ chain: similarity to von Willebrand Factor, fibronectin, actin, salivary proteins and aprotinin type protease inhibitors. *EMBO J.*, **9**, 385–393.
- Gay, S. and Miller, E.J. (1979) Characterization of lens capsule collagen: evidence for the presence of two unique chains in molecules derived from major basement membrane structures. *Arch. Biochem. Biophys.*, **198**, 370–378.
- Gay, S., Fine, J.D. and Storer, J.S. (1988) Autoantibodies to extracellular collagen matrix components in epidermolysis bullosa and other bullous diseases. *Arch. Dermatol. Res.*, **280**, 333–337.
- Hostikka, S.L. and Tryggvason, K. (1988) The complete primary structure of the $\alpha 2$ chain of human type IV collagen and comparison with the $\alpha 1(IV)$ chain. *J. Biol. Chem.*, **263**, 19488–19493.
- Janeczko, R.A. and Ramirez, F. (1989) Nucleotide and amino acid sequences of the entire human $\alpha 1(III)$ collagen. *Nucleic Acids Res.*, **17**, 6742.
- Jordan, R., Beeler, D. and Rosenberg, R. (1979) Fractionation of low molecular weight heparin species and their interactions with antithrombin. *J. Biol. Chem.*, **254**, 2902–2913.
- Keller, K.M., Keller, J.M. and Kuhn, K. (1986) The C-terminus of type I collagen is a major binding site for heparin. *Biochim. Biophys. Acta*, **882**, 1–5.
- Koda, J.E., Rapraeger, A. and Bernfield, M. (1985) Heparan sulfate proteoglycans from mouse mammary epithelial cells. *J. Biol. Chem.*, **260**, 8157–8162.
- Koliakos, G.G., Kouzi-Koliakos, K., Furcht, L.T., Reger, L.A. and Tsilibary, E.C. (1989) The binding of heparin to type IV collagen: domain specificity with identification of peptide sequences from the $\alpha 1(IV)$ and $\alpha 2(IV)$ which preferentially bind heparin. *J. Biol. Chem.*, **264**, 2313–2323.
- Kresina, T.F. and Miller, E.J. (1979) Isolation and characterization of basement membrane collagen from human placental tissue. Evidence for the presence of two genetically distinct collagen chains. *Biochemistry*, **18**, 3089–3097.
- Kuivaniemi, H., Tromp, G., Chu, M. and Prockop, D.J. (1988) Structure of a full-length cDNA clone for the prepro $\alpha 2(I)$ chain of human type I procollagen. *Biochem. J.*, **252**, 633–640.
- Laurent, T.C., Tengblad, A., Thunberg, L., Hook, M. and Lindahl, U. (1978) The molecular-weight-dependence of the anti-coagulant activity of heparin. *Biochem. J.*, **175**, 691–701.
- LeBaron, R.G., Hook, A., Esko, J.D., Gay, S. and Hook, M. (1989) Binding of heparan sulfate to type V collagen. *J. Biol. Chem.*, **264**, 7950–7956.
- Lee, M.K. and Lander, A.D. (1991) Analysis of affinity and structural selectivity in the binding of proteins to glycosaminoglycans: Development of a sensitive electrophoretic approach. *Proc. Natl. Acad. Sci. USA*, **88**, 2768–2772.
- Marcum, J. and Rosenberg, R. (1989) Role of endothelial cell surface heparin-like polysaccharides. In *Heparin and Related Polysaccharides*. Ann. NY Acad. Sci., **556**, 81–94.
- Miller, E.J. (1972) Structural studies on cartilage collagen employing limited cleavage and solubilization with pepsin. *Biochemistry*, **11**, 4903–4909.
- Miller, E.J. and Gay, S. (1987) Collagens. *Methods Enzymol.*, **144**, 3–41.
- Miller, E.J. and Rhodes, R.K. (1982) Preparation and characterization of the different types of collagen. *Methods Enzymol.*, **82**, 33–64.
- Myers, J.C., Loidl, H.R., Seyer, J.M. and Dion, A.S. (1985) Complete primary structure of the human $\alpha 2$ type V procollagen COOH-terminal propeptide. *J. Biol. Chem.*, **260**, 11216–11222.
- Piepkorn, M.W. and Chapman, D.L. (1985) Glycosaminoglycans and the substrate attachment of murine myeloma, 3T3, and cutaneous fibrosarcoma cells. *Lab. Invest.*, **53**, 22–29.
- Pihlajaniemi, T., Pohjolainen, E. and Myers, J.C. (1990) Complete primary structure of the triple-helical region and the carboxyl-terminal domain of a new type IV collagen chain, $\alpha 5(IV)$. *J. Biol. Chem.*, **265**, 13758–13766.
- Rapraeger, A., Jalkanen, M., Endo, E., Koda, J. and Bernfield, M. (1985) The cell surface proteoglycan from mouse mammary epithelial cells bears

- chondroitin sulfate and heparan sulfate glycosaminoglycans. *J. Biol. Chem.*, **260**, 11046–11052.
- Rosenberg, R.D., Jordan, R.E., Favreau, L.V. and Lam, L.H. (1979) Highly active heparin species with multiple binding sites for antithrombin. *Biochem. Biophys. Res. Commun.*, **86**, 1319–1324.
- San Antonio, J.D., Lander, A.D., Wright, T.C. and Karnovsky, M.J. (1992) Heparin inhibits the attachment and growth of Balb c/3T3 fibroblasts on collagen substrata. *J. Cell Physiol.*, **150**, 8–16.
- San Antonio, J.D., Slover, J., Lawler, J., Karnovsky, M.J. and Lander, A.D. (1993) Specificity in the interactions of extracellular matrix proteins with subpopulations of the glycosaminoglycan heparin. *Biochemistry*, **32**, 4746–4755.
- San Antonio, J.D., Lander, A.D., Karnovsky, M.J. and Slayter, H.S. (1994) Mapping the heparin binding sites on type I collagen monomers and fibrils. *J. Cell Biol.*, **125**, in press.
- Sanderson, R.D., Sneed, T.B., Young, L.A., Sullivan, G.L. and Lander, A.D. (1992) Adhesion of B lymphoid (MPC-11) cells to type I collagen is mediated by the integral membrane proteoglycan syndecan. *J. Immunol.*, **148**, 3902–3911.
- Sanderson, R.D., Turnbull, J.E., Gallagher, J.T. and Lander, A.D. (1994) Fine structure of heparan sulfate regulates syndecan-1 function and cell behavior. *J. Biol. Chem.*, **269**, in press.
- Soininen, R., Haka-Risku, T., Prockop, D.J. and Tryggvason, K. (1987) Complete primary structure of the $\alpha 1$ -chain of human basement membrane (type IV) collagen. *FEBS Lett.*, **225**, 188–194.
- Stamatoglou, S.C. and Keller, J.M. (1982) Interactions of cellular glycosaminoglycans with plasma fibronectin and collagen. *Biochim. Biophys. Acta*, **719**, 90–97.
- Su, M.W., Lee, B., Ramirez, F., Machado, M. and Horton, W. (1989) Nucleotide sequence of the full length cDNA encoding for human type II collagen. *Nucleic Acids Res.*, **17**, 9473–9474.
- Takahara, K., Sato, Y., Okazawa, N., Noda, A., Yaoi, Y. and Kato, I. (1991) Complete primary structure of human collagen $\alpha 1(V)$ chain. *J. Biol. Chem.*, **266**, 13124–13129.
- Tromp, G., Kuivaniemi, H., Stacey, A., Shikata, H., Baldwin, C.T., Jaenisch, R. and Prockop, D.J. (1988) Structure of a full-length cDNA clone for the prepro $\alpha 1(I)$ chain of human type I procollagen. *Biochem. J.*, **253**, 919–922.
- Tsilibary, E.C., Koliakos, G.G., Charonis, A.S., Vogel, A.M., Reger, L.A. and Furcht, L.T. (1988) Heparin type IV collagen interactions: equilibrium binding and inhibition of type IV collagen self assembly. *J. Biol. Chem.*, **263**, 19112–19118.
- Yaoi, Y., Hashimoto, K., Koitabashi, H., Takahara, K., Ito, M. and Kato, I. (1990) Primary structure of the heparin-binding site of type V collagen. *Biochim. Biophys. Acta*, **1035**, 139–145.

Received on November 19, 1993; revised on December 24, 1993; accepted on January 2, 1994