Binding of Heparan Sulfate to Type V Collagen

A MECHANISM OF CELL-SUBSTRATE ADHESION*

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The functions and molecular interactions of type V collagen in the pericellular matrix are unclear. Our studies show that type V collagen adsorbed on a surface binds heparin/heparan sulfate with apparent higher affinity than do collagen types I, II, III, IV, or VI, fibronectin, or laminin. Therefore, heparin-like molecules may mediate interactions between cells and type V collagen. Hence, type V collagen may act as an anchor for proteoglycans in the extracellular matrix and function as a substrate for glycosaminoglycan-mediated cell attachment. This model is supported by studies showing that Chinese hamster ovary (CHO) cell mutants which are deficient in glycosaminoglycan synthesis attach poorly to type V collagen substrates compared to wild-type cells, whereas attachment of CHO cell mutants to fibronectin substrates is not affected. Also, exogenous heparin reduces attachment of CHO, endothelial, and smooth muscle cells to type V collagen but does not affect cell attachment to fibronectin. The inhibitory activity of the exogenous heparin/heparan sulfate depends on the size and sulfate content of the polysaccharide chains. At tested concentrations, chondroitin sulfate does not affect the attachment of CHO cells or the binding of biotin-conjugated heparan sulfate to wells coated with type V collagen. These data suggest that a certain degree of structural specificity is involved in glycosaminoglycan binding to type V collagen.

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cell surface "receptors" which bind to defined sites in the matrix molecules. In fact, recent studies have shown that many adhesive matrix glycoproteins contain the amino acid sequence Arg-Gly-Asp (Ruoslahti and Pierschbacher, 1986) which is recognized by a class of matrix receptors referred to as the integrins (Hynes, 1987). The attachment and spreading of a cell on some extracellular matrix molecules appears to involve an interaction between an integrin receptor and an Arg-Gly-Asp sequence in the matrix protein. However, additional interactions between the cell and the matrix are also of importance. For example, formation of focal adhesions, which occur late in the adhesion process and represent specialized contacts between the cell and the extracellular matrix where stress fibers are anchored, requires two sets of receptor ligand interactions. In addition to an integrin-Arg-Gly-Asp interaction, a cell surface-associated heparan sulfate proteoglycan has to be stimulated by a glycosaminoglycan binding component to induce the formation of stress fibers and focal adhesions (Woods et al., 1986; LeBaron et al., 1988). A cell surface-associated heparan sulfate proteoglycan is also involved in the endocytosis of thrombospondin (Murphy-Ullrich and Mosher, 1987; Murphy-Ullrich et al., 1988), a process which appears to affect the migratory potential of the cell.

The observed importance of cell surface-associated heparan sulfate proteoglycans as receptors in the communication between cells and the extracellular matrix prompted us to analyze the binding potential of heparan sulfate to different matrix proteins. The data presented in this communication demonstrate that, of the proteins tested, type V collagen exhibits the highest apparent affinity for heparin/heparan sulfate. The specificity of this interaction is examined as well as its potential role in cell substrate adhesion.

MATERIALS AND METHODS

Ham's F-12 Growth Medium, Dulbecco's Modified Eagle's Medium, and trypsin (0.25%) were purchased from Gibco; fetal bovine serum and tissue culture flasks were from NUNC; Irvine Scientific, Santa Ana, CA; [35S]methionine (0.1-0.5 Ci/mmol) and aqueous counting scintillant were from Amersham Corp. Heparin, cycloheximide, D-biotin-N-hydroxysuccinimide ester and p-nitrophenyl phosphate were purchased from Sigma. Dextran sulfates and low molecular weight heparins were from Pharmacia LKB Biotechnology Inc., and Calbiochem, respectively. Heparin fractionated on a column of anti-antithrombin III coupled to Sepharose has been previously described (Höök et al., 1976). The method used for N-desulfation/N-acetylation of heparin has been described (Riesenfeld et al., 1980). Isolation and characterization of dermatan sulfate, bovine liver heparan sulfate (HS-I), and human aorta heparan sulfate (HS-II) have been de-

‡‡ J. Murphy-Ullrich and M. Höök, personal communication.

The abbreviations used are: HS-I, bovine liver heparan sulfate; HS-II, human aorta heparan sulfate; CHO, Chinese hamster ovary; SMC, smooth muscle cells; RAE, bovine aortic endothelial; BSA, bovine serum albumin; PBS, phosphate-buffered saline; CS, chondroitin sulfate; Hepes, 4-(2-hydroxethyl)1-piperazineethanesulfonic acid.

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scribed by Kjellén et al. (1980) and references therein. Bovine nasal cartilage chondroitin sulfate was provided by Dr. John Baker, Department of Biochemistry, University of Alabama at Birmingham. The uronic acid content of various polysaccharides was estimated by the method of Bitter and Muir (1962). Some of the glucosamine units in native heparin and heparan sulfate contain \(N\)-unsubstituted amino groups which can be detected with \(d\)-biotin-N-hydroxysuccinimide ester. Coupling of biotin to heparin and heparan sulfate was done according to the method of Orr (1981). Alkaline phosphatase-conjugated avidin was from Cooper Biomedical Inc., Malvern, PA. Hexapeptides with the sequences Gly-Arg-Gly-Glu-Ser-Pro and Gly-Arg-Gly-Asp-Thr-Pro, respectively (Dedhar et al., 1987), were provided by Dr. Erkki Ruoslahti, La Jolla Cancer Research Foundation, La Jolla, CA.

**Cell Culture**—Embryonic rat thoracic aorta smooth muscle cells (SMC) and Chinese hamster ovary (CHO) cells were obtained from the American Type Culture Collection (CRL 1476 and CCL 61, respectively). Bovine aortic endothelial (BAE) cells were provided by Dr. Joanne Murphy-Ullrich, Department of Biochemistry, UAB. SMC and BAE cells were maintained in Dulbecco's Modified Eagle's Medium containing high glucose (4.5 g/liter) supplemented with 2 mm glutamine and 20% v/v fetal bovine serum. CHO cell mutants defective in glycosaminoglycan synthesis were isolated and characterized (Esko et al., 1985, 1986, 1987, 1988) and maintained in Ham's F-12 medium supplemented with 10% fetal bovine serum (v/v), 100 units of penicillin G, and 100 \(\mu\)g of streptomycin sulfate/ml. All cells were used between the second and fourteenth passages. Cells were maintained routinely in monolayer cultures in an atmosphere of 100% relative humidity and 5% CO\(_2\) (CHO and BAE) or 10% CO\(_2\) (SMC). Cells were grown to near confluence, detached by trypsin treatment (CHO cells) or by trypsin treatment after rinsing the cells with PBS (Dulbecco and Vogt, 1954) containing 0.53 \(\mu\)M EDTA (BAE and SMC), and subsequently subcultured.

**Preparation of Substrates**—Fibronectin was purified from outdated human plasma (American Red Cross, Birmingham, AL) according to the method of Engvall and Ruoslahti (1977) as modified by Miekka et al. (1982). Human collagen types I and III (skin), type II (cartilage), and types IV, V, and VI (placenta) were isolated as described (Miller and Rhodes, 1982; Miller and Gay, 1987). Collagen stocks were kept in 0.5 N acetic acid at 4°C. For all experiments the collagens were diluted with or dialyzed against cold PBS. Tissue culture wells were coated with protein at the indicated concentration and incubated on an orbital shaker (90 rpm) for 24 h at 4°C followed by a PBS wash. Additional adsorption sites in the protein-coated wells were saturated by incubation with 2% BSA for 60 min at 4°C. In all experiments, BSA was heat-treated at 56°C for 60 min and filtered before use. The wells were rinsed twice with PBS and, when used in cell attachment assays, once with Buffer 3 (3.0 g of NaCl, 0.35 g of KCl, 0.16 g of MgSO\(_4\), 7\(\times\)H\(_2\)O, 0.18 g of CaCl\(_2\), 2\(\times\)H\(_2\)O, 2.4 g of Hepes, H\(_2\)O to 1 liter, pH 7.4) supplemented with 10 \(\mu\)g/ml cycloheximide and 60 \(\mu\)g/ml BSA. When 11-methyl labeled matrix proteins of known specific activities were used, the amounts of protein bound per cm\(^2\) were estimated to be 0.08, 0.2, 0.63, 0.2, 0.1, 0.1, 0.05, and 0.09 \(\mu\)g for collagen types I, II, III, IV, V, VI, fibronectin, laminin, respectively. Nonadhesive substrates were made by coating wells with 2% BSA.

**Polysaccharide-binding Assays**—Polysaccharides conjugated with biotin were diluted with PBS, added with or without competing polysaccharides to protein-coated microtiter wells, and incubated for 18 h at 4°C. The wells were rinsed, alkaline phosphatase-conjugated avidin in PBS was added, and the mixtures were incubated for 60 min at 4°C. After extensive rinsing the wells were incubated at 37°C with phosphatase substrate (p-nitrophenyl phosphate; Sigma 104 phosphatase substrate) and absorbance determined at 405 nm.

**Cell Attachment Assays**—Cells in monolayer cultures were radiolabeled with 0.125 \(\mu\)Ci/ml of \(^{35}\)S)methionine for 48–72 h. The cells were harvested by trypsin treatment, centrifuged, and resuspended in 30 ml of medium containing \(^{35}\)S)methionine. Cells were subsequently incubated at 37°C in suspension culture on an orbital shaker (170 rpm) for the indicated times to allow recovery from trypsin treatment. Cycloheximide (10 \(\mu\)g/ml) was added to suspension cultures 2 h before the attachment assay unless stated otherwise. Suspended cells were harvested by centrifugation, washed twice, and resuspended in Buffer 3. The number of cells was determined using a Coulter Counter (Coulter Electronics Inc., Hialeah, FL) or with a hemocytometer.

**RESULTS**

**Binding of Heparin and Heparan Sulfate to Collagens, Fibronectin, and Laminin**—A solid phase binding assay was developed to examine the heparin/heparan sulfate binding potential of various matrix proteins. Microtiter wells were coated with a fixed amount of various matrix proteins and subsequently incubated with biotin-conjugated heparin or heparan sulfate at increasing dilutions. The amount of bound conjugate was monitored by incubating the wells with phosphatase-conjugated avidin and, after extensive washing, with phosphatase substrate. In this assay, the relative affinities of different substrate proteins for different biotin-conjugated ligands can be compared. A higher affinity is reflected by significant substrate binding at a more diluted ligand concentration. By using this assay, type V collagen was found to have the highest apparent affinity for heparin/heparan sulfate relative to collagen types I, II, III, IV, and VI (Fig. 1). Types I, III, and VI collagen also bound significant amounts of heparin but with an apparent lower affinity than type V collagen. No binding of heparin to wells coated with type II or type IV collagen could be detected (Fig. 1a). It should be pointed out here that the apparent relative affinities of the different collagen types for heparin estimated by this assay depend on the amount of protein bound to the plastic wells during the coating procedure. Since the amount of type III collagen bound to the plastic is significantly lower than that of type V collagen (see "Materials and Methods"), the actual relative affinity of type III collagen for heparin is higher than that estimated in the assay.

In fact, when the experiments described in Fig. 1a were repeated using different batches of collagens, the binding of biotinylated heparin to type III collagen occasionally approached that of type V collagen (data not shown). It is tempting to speculate that in these cases the amount of type III collagen bound to the microtiter well was somewhat higher than that determined above.

![Fig. 1. Binding of heparin and heparan sulfate (HS-D) to collagen types I-VI, fibronectin, and laminin](image-url)
be detected at the concentrations of ligand tested.

Type V collagen were incubated with biotin-conjugated HS-I in the presence of increasing amounts of soluble collagen types 11, 111, IV, V, or VI. The results of this experiment show that soluble type V collagen inhibits the binding of heparan sulfate to wells coated with type I collagen. As indicated by the competition assay, soluble type V collagen also interacts with the polysaccharide. Heparin at relatively low concentrations (0.8 µg/ml or more) almost completely blocked the attachment of CHO cells to type V collagen substrates, whereas only 20-30% of added cells attached to a substrate of type V collagen. Under these conditions, <8% of cells attached to a substrate composed of BSA.

Attachment of cells to several matrix proteins including fibronectin (Pierschbacher and Ruoslahti, 1984), vitronectin (Hayman et al., 1985; Suzuki et al., 1985), and collagen type I (Dedhar et al., 1987) involve specific cell binding domains in the adhesive proteins which contain the amino acid sequence Arg-Gly-Asp. Also, the available amino acid sequence of the human a2 (V) collagen chain contains several Arg-Gly-Asp sequences (Myers et al., 1985). To examine if cell attachment to type V collagen involved a domain containing an Arg-Gly-Asp sequence, CHO cells were added to type V collagen-coated wells in the presence of the hexapeptide Gly-Arg-Gly-Asp-Thr-Pro (1 mg/ml). This peptide was previously shown to inhibit cell attachment to a Arg-Gly-Asp containing domain in type I collagen (Dedhar et al., 1987). Under the condition of the experiment, the Gly-Arg-Gly-Asp-Thr-Pro peptide did not have any inhibitory effect on the attachment of CHO cells to type V collagen substrates (data not shown) relative to a control peptide (Gly-Arg-Gly-Glu-Ser-Pro).

The role of cell-associated glycosaminoglycans in the attachment of CHO cells to type V collagen substrates was examined in experiments where the attachment assay was carried out in the presence of increasing amounts of soluble polysaccharides. Heparin at relatively low concentrations (0.8 µg/ml or more) almost completely blocked the attachment of CHO cells to wells coated with type V collagen, whereas heparin did not affect the attachment of cells to fibronectin-coated wells (Fig. 4).

These findings show that type V collagen can serve as a substrate for cell attachment and indicate that the cellular "receptors" involved in this interaction are not of the integrin type. Since cell attachment is inhibited by low concentrations of heparin, cell-associated heparan sulfate may be involved in the attachment process.
possibility that cell-associated glycosaminoglycans mediate the attachment of CHO cells to type V collagen, the time-dependent attachment of a set of CHO mutants deficient in different aspects of glycosaminoglycan biosynthesis was analyzed. CHO cells which express little (mutants 650 and 761) or no (mutant 745) glycosaminoglycans were unable to attach to a type V collagen substrate (Fig. 5). Cells that expressed about one-half the normal levels of chondroitin sulfate proteoglycans, but no heparan sulfate proteoglycans (mutant 605) were also unable to attach to type V collagen. In contrast, 60% of cells with normal levels of glycosaminoglycans (wild-type cells or mutant 605) attached to the type V collagen substrates (mutant 605 is deficient in sulfate transport but synthesizes fully sulfated proteoglycans using inorganic sulfate derived from sulfur-containing amino acids; Esko et al., 1986). No significant difference was observed between the attachment of wild-type and mutant cells to fibronectin substrates as described previously (LeBaron et al., 1988). These findings support the hypothesis that attachment of CHO cells to type V collagen substrates involves cell-associated heparan sulfate proteoglycans.

Specificity of the Binding of Heparan Sulfate and CHO Cells to Type V Collagen Substrate—To examine the specificity of the binding of biotin-conjugated heparan sulfate and the attachment of CHO cells to wells coated with type V collagen, experiments were carried out in the presence of different well-characterized polysaccharides as potential inhibitors. In initial experiments, CHO cells were seeded in type V collagen-coated wells in the presence of increasing concentrations of heparin, heparan sulfate (HS-I and HS-II), or chondroitin sulfate (Fig. 6). The presence of heparin or heparan sulfates markedly inhibited the attachment of CHO cells to type V collagen-coated substrates, whereas chondroitin sulfate was without effect. However, the inhibitory activity of the different heparan sulfate polysaccharides varied. A lower concentration of the highly sulfated polysaccharide heparin (sulfate content greater than 2 residues/disaccharide) was required to obtain 50% inhibition of cell attachment compared to that of the heparan sulfate preparation HS-I which has an intermediate sulfate content (0.87 residues/disaccharide). An even higher concentration of the poorly sulfated heparan sulfate HS-II (0.66 sulfate residues/disaccharide) was required for a comparable inhibition of cell attachment.

Similarly, the binding of biotin-derivatized HS-I to type V collagen was inhibited in a concentration-dependent manner by the more highly sulfated polysaccharides, HS-I and heparin. Little, if any, inhibition was observed in the presence of the less sulfated polysaccharide, HS-II, or by chondroitin sulfate (Fig. 7). The data indicate that the binding of heparan sulfate to type V collagen substrates is correlated to the sulfate content of the polysaccharide. It is noteworthy that the presence of chondroitin sulfate did not affect the attachment of CHO cells or the binding of HS-I to type V collagen substrates despite the face that the sulfate content of this glycosaminoglycan (1 residue/disaccharide) is substantially higher than that of HS-II.

In further experiments, the inhibitory activity of additional polysaccharides were examined. The binding of biotin-derivatized HS-I and the attachment of CHO cells to type V collagen substrates exhibited essentially identical sensitivity to the different inhibitory polysaccharides (Fig. 8). Hence, unfractionated heparin and heparin fractionated on an anti-thrombin-Sepharose column into high affinity and low affinity species had the same high degree of inhibition in both
jugated HS-I binding and CHO cell attachment to type V collagen were preincubated with the indicated concentrations of the polysaccharide for 60 min at 4 °C followed by addition of biotin-conjugated HS-I and further incubation at 4 °C for 4 h. The wells were washed and incubated with alkaline phosphatase-conjugated avidin at 4 °C for 4 h. After washing, phosphatase substrate was added, and the wells were incubated at 37 °C and absorbance determined at 405 nm. Nonspecific binding of biotin-conjugated HS-I to wells coated with type V collagen (data not shown). The presence of heparin-like polysaccharides was reduced as the sulfate content of the polysaccharide was lowered. HS-I exhibited a reduced inhibition activity compared to heparin, and HS-II showed essentially no activity at the concentration used in the two assays. Of the galactosaminoglycans, chondroitin sulfate (CS) had essentially no inhibitory activity, whereas dermatan sulfate inhibited the attachment of CHO cells to substrates of type V collagen but did not affect the binding of biotin-conjugated heparan sulfate to wells coated with type V collagen. Dextran sulfate with a high sulfate content (2.2 residues/disaccharide) also inhibited the binding of biotin-conjugated heparan sulfate to wells coated with type V collagen.

Assays. Low molecular weight heparins showed decreased inhibitory activity as a function of decreasing molecular size to 5000 and 3000. N-Desulfation of heparin resulted in a dramatic drop in inhibitory efficiency, an activity which was partly restored by acetylation of exposed amino groups. Consistent with the data shown in Fig. 7, the inhibitory activity

of the heparin-like polysaccharides was reduced as the sulfate content of the polysaccharide was lowered. HS-I exhibited a reduced inhibition activity compared to heparin, and HS-II showed essentially no activity at the concentration used in the two assays. Of the galactosaminoglycans, chondroitin sulfate (CS) had essentially no inhibitory activity, whereas dermatan sulfate inhibited the attachment of CHO cells to substrates of type V collagen but did not affect the binding of biotin-conjugated heparan sulfate to wells coated with type V collagen. Dextran sulfate with a high sulfate content (2.2 residues/disaccharide) also inhibited the binding of biotin-conjugated HS-I and the attachment of CHO cells to wells coated with type V collagen (data not shown).

Attachment of Cells of the Vessel Wall to Type V Collagen Substrates—Type V collagen is found in the extracellular matrix of a wide variety of tissues (Miller and Gay, 1987). In the blood vessel wall it is associated with the pericellular matrix of smooth muscle and endothelial cells (Gay et al., 1981), and type V collagen has been found in increasing amounts in human atherosclerotic plaques (Ooshima, 1981). Because endothelial cells and smooth muscle cells in vivo are surrounded by a type V collagen matrix and as most cells contain membrane-associated proteoglycans, we examined the ability of BAE cells and SMC to attach to type V collagen substrates in our in vitro assay. Initial experiments showed that both cell types attached to wells coated with type V collagen in a reaction that depended on incubation time and substrate concentrations (data not shown). The presence of low concentrations of heparin significantly reduced the number of both SMC and BAE cells attaching to the type V collagen substrates but had no effect on cell attachment to fibronectin (Fig. 9, a and b). Hence, cell-associated glycosaminoglycans also seem to mediate the attachment of SMC and BAE cells to type V collagen substrates. However, heparin

![Fig. 7. Polysaccharide-mediated inhibition of HS-I binding to type V collagen.](image)

![Fig. 8. Polysaccharide-mediated inhibition of biotin-conjugated HS-I binding and CHO cell attachment to type V collagen substrates.](image)

![Fig. 9. Inhibition by heparin of BAE cell, SMC, and CHO cell attachment to fibronectin and type V collagen substrates.](image)
could not completely inhibit the attachment of SMC or BAE cells to type V collagen, whereas the attachment of CHO cells to type V collagen was reduced below that observed on BSA (Fig. 9c). The presence of the Gly-Arg-Gly-Asp-Thr-Pro peptide at 1 mg/ml did not affect the attachment of SMC or BAE cells to type V collagen substrates (data not shown).

**DISCUSSION**

The results of the solid phase binding assays indicate that type V collagen has a higher affinity for heparin/heparan sulfate than most other collagens, fibronectin, or laminin. An interaction between the protein and cell surface-associated heparan sulfate appears to mediate the attachment of CHO cells to type V collagen substrates since (a) wild-type but not heparan sulfate-deficient mutant cells attach to wells coated with type V collagen and (b) heparin at low concentrations (≥ 0.1 μg/ml) inhibits the attachment of wild-type CHO cells to substrates of type V collagen.

The polysaccharide specificity of these interactions was studied in experiments where well defined, unlabeled polysaccharides were examined as potential inhibitors. Binding of biotin-conjugated HS-I and attachment of wild-type CHO cells to wells coated with type V collagen show similar sensitivity to added polysaccharides. The apparent affinity for type V collagen increases as the sulfate density and the molecular size of the heparin-like polysaccharides increase. Heparin-like polysaccharides are preferred over galactosaminoglycans, but highly sulfated dextran sulfates show inhibitory activity. Dermatan sulfate and aorta heparan sulfate (HS-II) partly inhibit the attachment of CHO cells but have no effect on the binding of biotin-conjugated HS-I to wells coated with type V collagen. These results may reflect a difference in sulfate content between bovine liver heparan sulfate (HS-I) and CHO cell heparan sulfate which appears to have a sulfate content comparable to that of HS-II (Bame and Esko, 1989).

Although the results reported here define the structural requirements of a type V collagen-binding glycosaminoglycan in general terms, they do not define the chemical structure of the binding site. A precise requirement in the monosaccharide sequence and sulfate substitution pattern may exist or may heparin sequences may bind to type V collagen provided they are sufficiently long. In the latter case, the affinity of the heparin sequence for type V collagen may be determined largely by the sulfate content of the interacting sequence. Recent studies of the conformation of monosaccharide units in glycosaminoglycans suggest that the introduction of sulfated iduronic acid residues in a polysaccharide will substantially increase the flexibility of the polymer since this unit can occur in several conformations (C1, C4, or S) depending on parameters such as the position of iduronic acid residues in the glycosaminoglycan and the presence or position of sulfate residues on the sugars (reviewed in Casu et al., 1988).

Cell-associated glycosaminoglycans have previously been shown to participate in cell adhesion phenomena. A heparan sulfate proteoglycan seems to be required for the formation of focal adhesions and stress fibers (Woods et al., 1986; LeBaron et al., 1988) and in the assembly of fibronectin fibrils (Woods et al., 1988). Also, a heparan sulfate/chondroitin sulfate hybrid proteoglycan can mediate the attachment of mammary epithelial cells to type I collagen (Koda and Bernfield, 1984). However, a receptor of the integrin type has also been shown to mediate cell adhesion to type I collagen where an Arg-Gly-Asp-Thr sequence is the recognition site in the collagen molecule (Dedhar et al., 1987). Other surface molecules have also been reported to act as type V collagen receptors including a cell surface glycoconjugate (Grotendorst et al., 1981) and cell surface components shown to bind to a cyanogen bromide-derived peptide of the α2 (V) chain (Leushner and Haust, 1985).

Cell-associated heparan sulfate appears to be the only surface component capable of mediating CHO cell attachment to type V collagen substrates. Low concentrations of heparin completely block attachment of these cells to type V collagen substrates. Heparan sulfate-deficient CHO mutant cells show no residual attachment to type V collagen substrates although these cells attach to substrates of type I, type III, type IV, and type VI collagen.

The attachment of BAE cells and the rat SMC to type V collagen substrates are markedly inhibited by low concentrations of heparin. This indicates that cell-associated heparan sulfate is an important receptor in the adhesion of these cells to type V collagen. However, even in the presence of 100 μg/ml of heparin, a significant number of these cells attach to the type V collagen substrate. This indicates that BAE cells and SMC may express additional cell surface molecules which mediate attachment to type V collagen. An exogenous peptide with the sequence Gly-Arg-Gly-Asp-Thr-Pro (Dedhar et al., 1987) does not inhibit cell attachment to type V collagen suggesting that these receptors have a substrate specificity different from the integrin-like receptors which mediate cell attachment to type I collagen. Therefore it is possible that type V collagen contains additional cell binding site(s) which may not involve an Arg-Gly-Asp sequence.

In the vessel wall, type V collagen is closely associated with the cell surface. The role(s) that heparan sulfate proteoglycans play in this association and in the integrity of the extracellular matrix of the vessel wall remains to be clarified.

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