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Interaction of Skeletal Muscle Cells With Collagen Type IV Is Mediated by Perlecan Associated With the Cell Surface

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Abstract We have previously shown that the expression of perlecan, a heparan sulfate proteoglycan localized on the myoblast surface, is down-regulated during terminal differentiation of skeletal muscle myoblasts (Larraín et al. [1997] Exp. Cell Res. 234:405–412). In this study, we have evaluated the biochemical characteristics of perlecan, its association with the myoblast surface, and its involvement in C_2C_{12} myoblast adhesion to different substrates. Perlecan associated with myoblasts was solubilized by Triton X-100, whereas heparin, high salt, and RGD peptides were unable to solubilize perlecan. Pre-incubation of myoblasts with [^{35}S]-Na_2SO_4, followed by solubilization with Triton X-100 and immunoprecipitation with antibodies against murine perlecan, demonstrated that this proteoglycan present on the cell surface has a heterogeneous size profile with a K_{av} value of 0.45, determined by Sepharose CL-4B chromatography. Myoblasts were found to adhere with decreasing affinities to collagen type IV, type I, laminin, fibronectin, perlecan, and matrigel. We found that cell adhesion to collagen type IV was inhibited by blocking this substrate with exogenous perlecan prior to cell plating, whereas no effect was observed for laminin. Furthermore, adhesion of myoblasts to collagen type IV was inhibited by the perlecan core protein obtained by treatment of perlecan with heparitinase, as well as by pre-incubation of the cells with antibodies against murine perlecan. These data support the idea that skeletal muscle cells interact with collagen type IV through the perlecan core protein present on the surface of undifferentiated myoblasts. J. Cell. Biochem. 75:665–674, 1999.

Key words: perlecan; myoblast; collagen type IV; cell adhesion; heparan sulfate proteoglycans

Cell surface heparan sulfate proteoglycans are found almost ubiquitously on the cell surface and in the extracellular matrix (ECM) of mammalian cells. These macromolecules interact with a great variety of ligands, including ECM constituents, adhesion molecules, and growth factors [Bernfield et al., 1992; Carey, 1997], indicating that the presence of heparan sulfate proteoglycans on the cell surface may influence the response of a particular cell type to changes in the environment. Perlecan, first isolated from the murine Engelbreth-Holm-Swarm (EHS) tumor [Hassell et al., 1980; Noonan et al., 1991; Noonan and Hassell, 1993], is an intrinsic heparan sulfate-proteoglycan and a constituent of basement membranes and ECMs, for which several functions have been reported. Perlecan has been implicated in the formation of a selective charge barrier that regulates the filtration of solutes in renal glomeruli [Kanwar and Farquhar, 1979; Kanwar et al., 1980], in the binding to several ECM components, such as laminin, collagen type IV, and fibronectin [Yamagata et al., 1993; Mayer et al., 1997; Brown et al., 1997], has been shown to possess activity as a low-affinity receptor for basic fibroblastic growth factor (FGF-2), a potent angiogenic modulator [Aviezer et al., 1994a], and to participate as a cell adhesion molecule [Gauer et al., 1996; Hayashi et al., 1992].

The primary structure of perlecan consists of at least five separate domains (I–V) and includes a putative heparan sulfate-binding re-

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gion in domain I. Domain II is homologous to the low-density lipoprotein-receptor, whereas domain III consists of three cysteine-free laminin-like globules. In addition, murine domain III also contains an integrin-binding sequence arg-gly-asp (RGD), located in its second globular subdomain [Noonan et al., 1991]. Domain IV contains variably spliced immunoglobulin-type repeats similar to those found in neural adhesion molecule. Domain V at the C-terminus of perlecan consists of three cysteine-free neurexin-like globules that alternate with epidermal growth factor-like units and is most homologous to the equivalent C-terminal region of agrin [Appella et al., 1988]. This multidomain structure is responsible for the multiple functions described for perlecan [Iozzo, 1994; Murdoch and Iozzo, 1993; Timpl, 1994].

It has been demonstrated that perlecan supports the attachment of various types of cells [Clement et al., 1989; Clement and Yamada, 1990]. For instance, murine perlecan has been shown to support the adhesion of aortic endothelial cells via its intact core protein, an interaction that could be partially inhibited by the RGD synthetic peptide as well as an anti-β1 integrin antibody [Hayashi et al., 1992]. Similarly, the recombinant domain III of perlecan was found to promote cell attachment through its RGDS sequence [Chakravarti et al., 1995].

We have previously demonstrated that in skeletal muscle cells, the expression of perlecan is down-regulated during terminal differentiation [Larrain et al., 1997a]. Through immunolocalization studies, perlecan was found to be localized on the myoblast surface. By immunogold staining, we were able to demonstrate that it is associated with patches of an incipient ECM in close contact with the plasma membrane [Larrain et al., 1997a]. The precise function for perlecan on the surface of myoblast cells is unknown, although there is evidence to suggest that perlecan may be important for myogenesis. In *Caenorhabditis elegans,* the product of the unc-52 gene corresponds to a truncated form of the perlecan core protein [Gilchrist and Moerman, 1992; Rogalski et al., 1993]. Mutations in this gene have been found to cause disruption of the skeletal muscle architecture, and to generate a disorganized body wall muscle phenotype, suggesting that perlecan-like molecules are involved in maintaining the structural integrity of skeletal muscle. Other possible functions are suggested by the finding that in some cells, the presence of perlecan is necessary for FGF-2 presentation, a growth factor required for mitogenic activity and also a strong inhibitor of terminal skeletal muscle differentiation [Brunetti and Goldfine, 1990; Aviezer et al., 1994a]. Moreover, it has been shown that stable expression of perlecan antisense cDNA leads to the suppression of the autocrine and paracrine functions of FGF-2 in fibroblasts [Aviezer et al., 1997]. In addition to a role in growth factor signaling, perlecan might be required for the adhesion of myoblasts to their substrata, a function likely to be critical during the development and formation of skeletal muscle [Molkentin and Olson, 1996], as well as during skeletal muscle regeneration [Cannon and St. Pierre, 1998]. In this study, we have investigated the association of cell surface perlecan with myoblasts, analyzed the biochemical characteristics of perlecan associated to the myoblast surface and examined the involvement of perlecan in myoblast adhesion to ECM constituents. We report that perlecan is a polydisperse heparan sulfate proteoglycan that can be solubilized from the cell surface using a nonionic detergent, but not with high-ionic-strength or heparin buffers, and that adhesion of myoblasts to collagen type IV is dependent on the presence of perlecan on the myoblast surface.

MATERIALS AND METHODS

Materials

[^{35}S]-Na_2SO_4, carrier free and [^{35}S]-methionine (Protein labeling mix; 1,175 Ci/mmol) were obtained from New England Nuclear (Boston, MA). Protein A-Sepharose, DEAE-Sephacel, Sepharose CL-4B, fibronectin, collagen types I and IV, and laminin were purchased from Sigma Chemical Co. (St. Louis, MO). Perlecan and matrigel were obtained from Collaborative Research (Bedford, MA). C_2C_{12} cells, a myogenic cell line isolated from mouse skeletal muscle [Yaffe and Saxel, 1977], was obtained from American Type Culture Collection (MD). The lactate dehydrogenase (LDH) assay kit was obtained from Promega (Madison, WI). Other reagents were obtained from commercial sources.

Methods

Cell cultures. The mouse skeletal muscle cell line, C_2C_{12} was grown as described [Brandan et al., 1991; Larrain et al., 1997b]. Cells were plated at a density of 4,000 cells/cm^2 in Dulbecco's modified Eagle's medium (DMEM) with 1 g/L glucose supplemented with 10% fetal

calf serum (FCS) (Sigma) and 0.5% chick embryo extract (GIBCO-BRL, Gaithersburg, MD) and incubated at 37°C in a humidified 5% CO_2 atmosphere for 48 h.

Sequential solubilization of perlecan from the cell surface of myoblasts. Myoblasts grown to 80% confluence were first washed three times with phosphate-buffered saline (PBS) and incubated with PBS containing 10 mM phytic acid followed by PBS with either 2.0 mg/ml heparin, 1.0 mg/ml of RGD peptide, 1.0 M NaCl, or 2.0 mg/ml heparin and 1.0 M NaCl for 10 min at 4°C. Cells were then washed three times with cold PBS, fixed with 3% paraformaldehyde for 30 min at room temperature, and finally rinsed with 5% nonfat milk in PBS (Blotto) and incubated with antibodies against murine perlecan [Hassell et al., 1980; Ledbetter et al., 1985]. LDH was assayed following the manufacturer instructions.

Immunolocalization studies. For staining of extracellular perlecan, C_2C_{12} cells were incubated with antibodies against murine perlecan [Hassell et al., 1980; Ledbetter et al., 1985] diluted 1:50 in Blotto for 1 h at 4°C before fixation [Larrain et al., 1997a]. After rinsing, cells were fixed with 3% paraformaldehyde for 30 min at room temperature, rinsed with Blotto, and incubated for 1 h at room temperature with affinity purified fluorescein-conjugated secondary antibodies diluted in Blotto. After rinsing, the plates and the cover slips were mounted and viewed under a Nikon Diaphot-inverted microscope equipped for epifluorescence [Melo et al., 1996].

Labeling of cultures and analysis of proteoglycans. C_2C_{12} myoblasts were seeded onto 35-mm dishes and radiolabeled by the addition of medium containing 100 µCi/ml [^{35}S]-Na_2SO_4 for 18 h. Cells were then extracted using the indicated Triton X-100 concentrations in PBS. [^{35}S]-Na_2SO_4 incorporation into macromolecules was evaluated by cetyl pyridinium chloride (CPC) precipitation [Brandan and Inestrosa, 1987; Brandan et al., 1996].

Gel filtration chromatography. Detergent-solubilized material was fractionated on a DEAE-Sephacel column pre-equilibrated in 10 mM Tris-HCl pH 7.5, 0.2 M NaCl, 0.1% Triton X-100 and eluted using a linear NaCl gradient (0.2–1.0 M) dissolved in the same solvent at a flow rate of 5 ml/h. Fractions (1.0 ml) were collected and radioactivity and conductivity determined using a conductimeter. Pooled fractions were resolved using an analytical Sepharose CL-4B column (100 cm × 1 cm) prepared in 1% sodium dodecyl sulfate (SDS), 0.1 M NaCl, 50 mM Tris-HCl buffer, pH 8.0. Samples (0.5 ml) were applied to the column together with previously fractionated blue Dextran (2000) and phenol red to mark void and total volumes, respectively. Columns were eluted at a flow rate of 5.0 ml/h, and effluent fractions of 0.8 ml were collected and aliquots counted for radioactivity.

Immunoprecipitation of perlecan. Heparan sulfate proteoglycans separated on a DEAE-Sephacel column were immunoprecipitated with protein A-Sepharose coated with antibodies against murine perlecan. Briefly, [^{35}S]-sulfate-labeled samples were first incubated with nonimmune serum, followed by incubation with protein A-Sepharose to remove nonspecific binding. Then, samples were incubated with anti-perlecan antibodies and the precipitates harvested after incubation with protein A-Sepharose.

Enzymatic treatments. Aliquots of pooled fractions obtained from the DEAE-Sephacel column or perlecan were incubated with 5 milliunits of heparitinase (Seikagaku Co, Japan) in 10 mM Tris, pH 7, 0.1 mM $CaCl_2$ for 3 h at 37°C [Larrain et al., 1997a]. Chondroitinase ABC treatment of pooled samples was carried out as described previously [Brandan et al., 1991], as was nitrous acid treatment [Brandan and Inestrosa, 1987].

Cell attachment assay. Myoblast attachment to substrate-bound macromolecules was assayed as follows: 80% confluent myoblasts were first labeled with [^{35}S]-methionine for 2 h. Plates were washed with PBS and then extracted using 0.5 mM EDTA in PBS for 20 min at 37°C. Cells were harvested and plated at a density of 2,000/cm^2 in 24-well plates (2 cm^2/well) (Costar, Cambridge, MA) coated with either 2 µg/cm^2 laminin, 5 µg/cm^2 fibronectin, 10 µg/cm^2 collagen type I, 10 µg/cm^2 collagen type IV, 1.0 µg/cm^2 perlecan or perlecan core. As blank wells were coated with 10 µg/cm^2 bovine serum albumin (BSA). The fraction of bound substrate, on the basis of mass, was found to be nearly identical when wells were incubated with these concentrations as measured with radioionidated substrate. Cells were incubated at 37°C in serum-free DMEM for 10 min, after which dishes were washed three times with PBS and extracted with 1% SDS in 10 mM Tris-HCl, pH 7.5. The number of attached cells was calculated by determining the radioactiv-

ity of aliquots of solubilized material and correlating this with a standard curve of radioactive counts versus number of cells.

RESULTS

Association of Perlecan With Myoblast Surface

We have previously shown that perlecan is present on the myoblast cell surface [Larrain et al., 1997a]. To evaluate the type of association involved, myoblast cultures were incubated with various solubilizing agents in order to establish whether this interaction could be disturbed. When cells were incubated with 1 mg/ml of soluble RGD peptide, and the presence of perlecan evaluated by immunostaining, a surface distribution similar to that of control cells was observed (Fig. 1B). Treatment of the cells with either ionic strength (1.0 M NaCl; Fig. 1C), 2.0 mg/ml of heparin (Fig. 1D), or a combination of both (Fig. 1E), was unable to solubilize perlecan from the myoblast surface. Figure 1F shows total absence of cell immunostaining in the absence of perlecan antibodies. Treatment with ionic strength or heparin released a significant

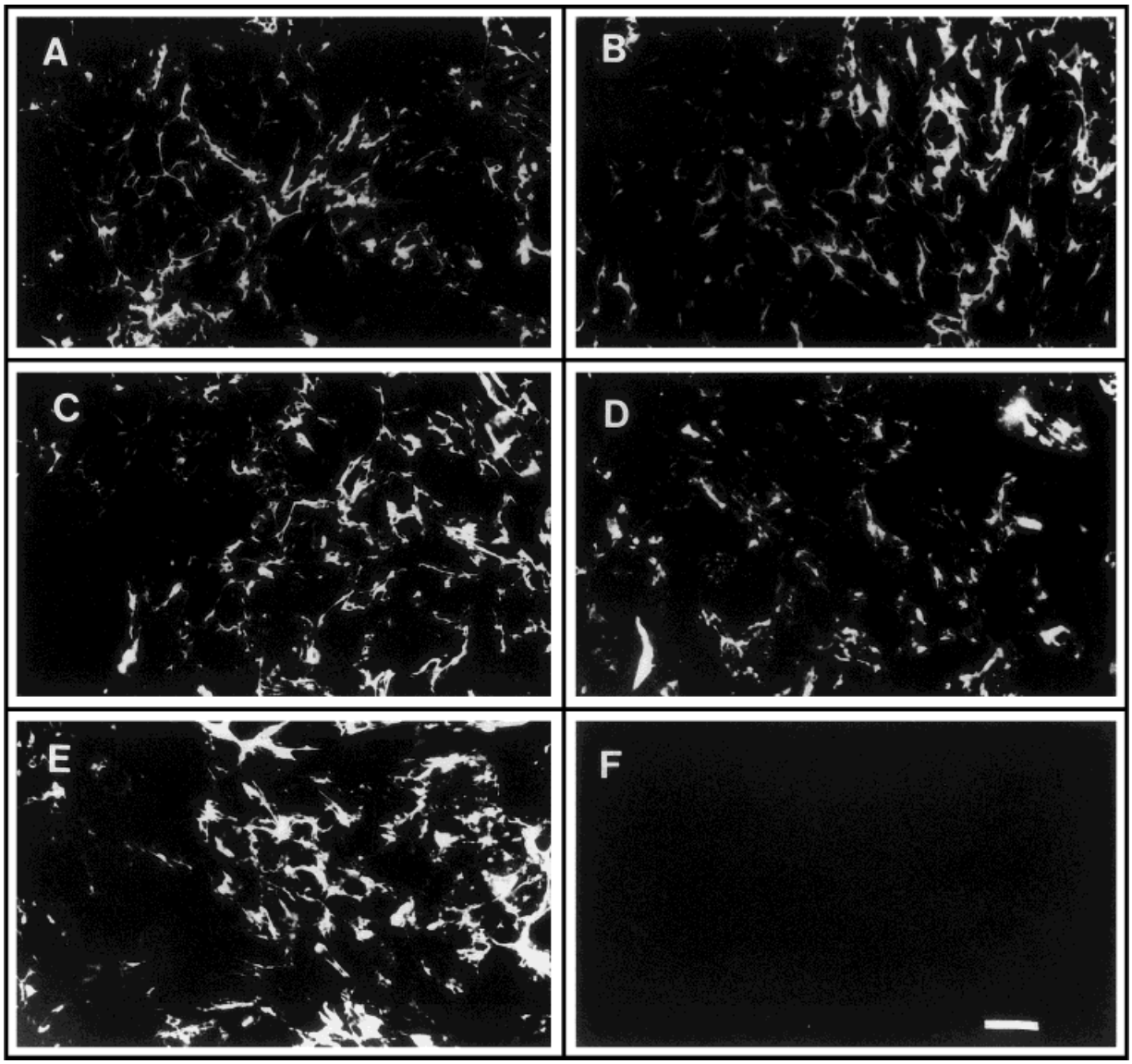

Fig. 1. Perlecan is not solubilized by RGD peptides, high ionic strength, and heparin. Myoblasts were grown on coverslips for 3 days and incubated with phosphate-buffered saline (PBS) **(A)**, 1.0 mg/ml of soluble RGD peptide **(B)**, 1.0 M NaCl **(C)**, 2.0 mg/ml heparin **(D)**, 1.0 M NaCl, and 2.0 mg/ml heparin **(E)**. Cells were then processed for indirect immunofluorescence staining with anti-perlecan antibodies, and secondary antibodies conjugated to fluorescein. **F:** Antibodies against perlecan were omitted. Scale bar = 15 μm.

amount of proteins from the cell culture, evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (data not shown), without an effect on perlecan release. To evaluate whether perlecan association to the myoblast cell surface was sensitive to nonionic detergent myoblasts were incubated with increasing concentrations of Triton X-100, and then immunostained. Figure 2A–E indicates that increasing concentrations of Triton X-100 solubilized perlecan from the surface, with a maximal effect at 0.1% Triton X-100. When the same cells were viewed by phase-contrast microscopy the increasing absence of cell membrane structure became evident and nuclei could be seen attached to the culture dish (Figs. 2F–J). The solubilization profile of LDH, a cytosolic protein, was also analyzed as a function of Triton X-100 concentration, and was found to be highest at 0.05% Triton X-100 (Fig. 3), indicating that the cell membrane was solubilized. These results indicated that perlecan was associated to the myoblast surface, through an interaction that was independent of the RGD sequence, and insensitive to either ionic strength or heparin, yet sensitive to nonionic detergent, suggesting a direct or nondirect association of perlecan with the cell membrane.

Biochemical Characterization of Perlecan Synthesized by Myoblasts

To determine the biochemical characteristics of perlecan associated to myoblasts, Triton X-100 extracts of radiolabeled cells were applied to a DEAE-Sephacel column and eluted using a 0.2–1.0 M NaCl gradient. Figure 4A shows that radioactive material eluted in three peaks at NaCl concentrations of 0.3, 0.45 and 0.65 M, respectively. The proteoglycans eluting at 0.3 and 0.45 M NaCl were totally sensitive to nitrous acid treatment, indicating their heparan sulfate proteoglycan nature, whereas those eluting at 0.65 M NaCl were insensitive to nitrous acid treatment and totally degraded by chondroitinase ABC, suggesting a chondroitin/dermatan sulfate proteoglycan content (data not shown). When these peaks where pooled and then immunoprecipitated with antisera against murine perlecan, perlecan was found to be present mainly in the peak eluting at 0.45 M NaCl. To characterize the relative hydrodynamic size of the proteoglycans present in this perlecan containing fraction, the 0.45 M NaCl peak was subjected to analytical Sepharose CL-4B chromatography, from which two main peaks with K_{av} values of 0.45 and 0.75 were resolved (Fig. 4B). When the 0.45 M NaCl peak (Fig. 4A) was immunoprecipitated with anti-perlecan antibodies and then fractionated on a Sepharose CL-4B column a polydisperse material which eluted with a K_{av} value of 0.45 corresponding to perlecan was observed. These results indicated that myoblasts synthesize perlecan which possessed a polydisperse heparan sulfate nature, and eluted from Sepharose CL-4B with a K_{av} value of 0.25–0.6 K_{av}.

Perlecan Is Involved in Myoblast Adhesion to Collagen Type IV

The surface location of perlecan on myoblast surface implies that it may be involved in myoblast cell adhesion. The attachment of radiolabeled myoblasts was investigated using several adhesive proteins of the ECM, including perlecan. Myoblasts were found to bind the following surfaces with decreasing affinities: collagen type IV, laminin, collagen type I, fibronectin, matrigel, and perlecan (Fig. 5). To determine that the different adhesive proteins bind to the dish surface, indirect specific immunostaining was performed for collagen type IV, fibronectin, laminin, and perlecan (data not shown). Adhesion could not be further increased by raising coating concentrations of >10 μg/cm^2 for collagen types I and IV and fibronectin, or 5 μg/cm^2 for laminin, perlecan, and matrigel. To evaluate further the participation of perlecan in myoblast adhesion, plastic dishes were coated with either collagen type IV or laminin, followed by incubation in the presence or absence of perlecan before the cells were plated. The presence of perlecan was found to inhibit almost completely the adhesion of myoblasts to collagen type IV, without affecting their adhesion to laminin (Fig. 6). Control experiments indicated that perlecan binds equally well to dishes coated with collagen type IV or laminin as determined by indirect immunolocalization of perlecan (data not shown). In order to determine whether this interaction was mediated by the heparan sulfate side chains or the core protein of perlecan, this proteoglycan was treated with heparitinase before its incubation with the substrata collagen type IV. Figure 7A shows that both intact and deglycosylated perlecan were able to inhibit almost completely the attachment of myoblasts to collagen type IV. This interaction was also inhibited by 85% when myoblasts were

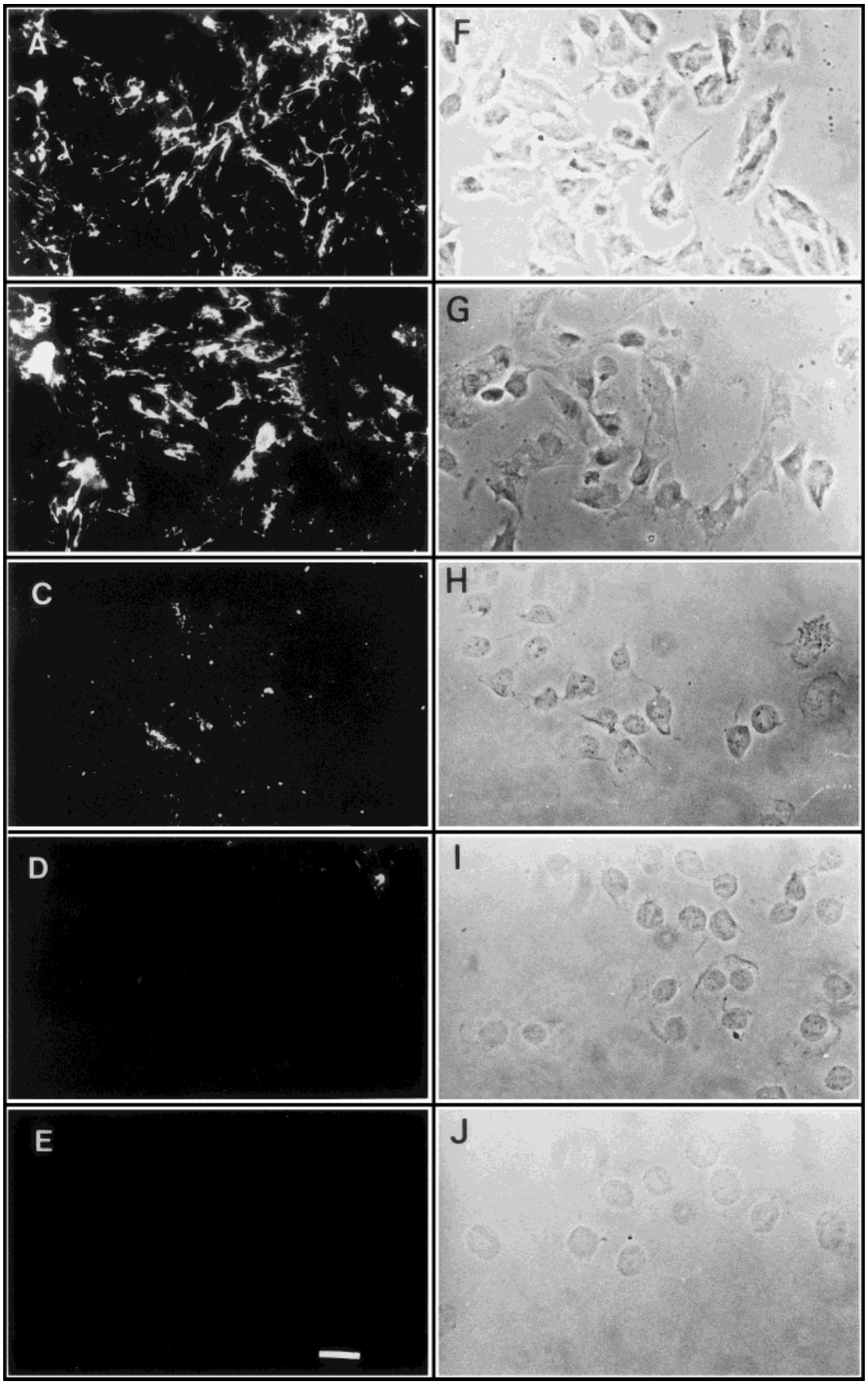

Figure 2.

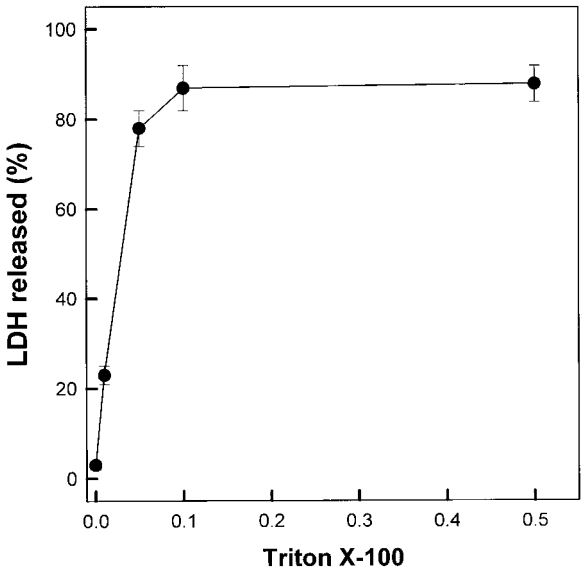

Fig. 3. Detergent treatment of plated myoblasts released almost 90% of total lactate dehydrogenase activity. Myoblasts were incubated with the indicated Triton X-100 concentrations, and the release of cytosolic lactate dehydrogenase (LDH) was determined in the soluble material. Results are presented as percentage of maximal LDH activity determined in total myoblasts. Values are the mean and standard deviation of three experiments.

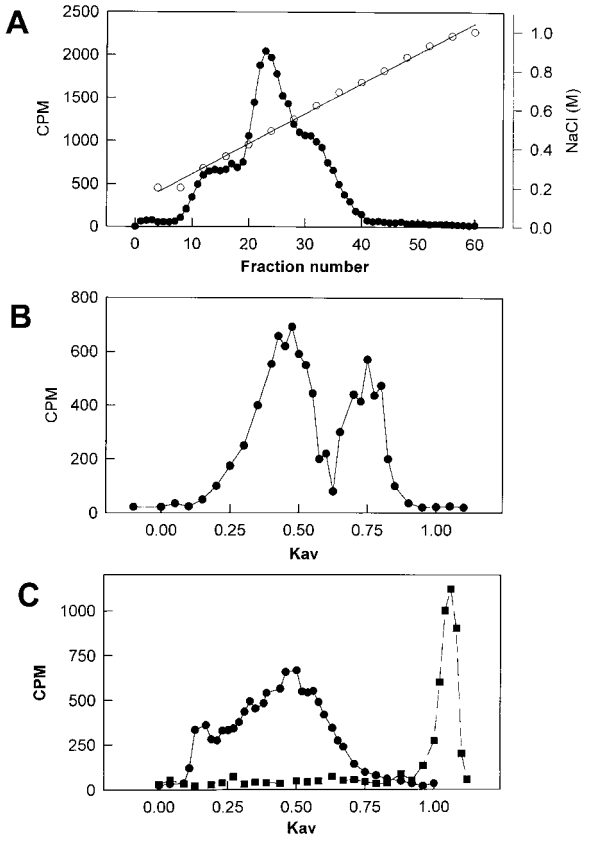

Fig. 4. Biochemical characterization of perlecan solubilized by Triton X-100 from myoblasts. Myoblasts were incubated for 18 h in medium containing [^{35}S]-Na_2SO_4 and then solubilized with 0.1% Triton X-100. **A:** The extract was applied to a DEAE-Sephacel column and eluted using a linear NaCl gradient. **B:** Pooled fractions that eluted at 0.45M NaCl in **A** were concentrated and fractionated through a Sepharose Cl-4B column and resolved into two main peaks with K_{av} values of 0.45 and 0.75. **C:** The same pooled fraction obtained in **A** was immunoprecipitated using anti-perlecan antibodies and the immunoprecipitate directly fractionated through a Sepharose CL-4B column (circles) or treated with heparitinase before Sepharose CL-4B chromatography (squares).

pre-incubated with antisera against perlecan and then seeded onto a collagen type IV-coated surface (Fig. 7B). As a control, no effect in myoblast attachment was observed when cells were pretreated with nonimmune antibodies. These results suggested that perlecan localized on the surface of myoblasts participates in the adhesive properties of these cells to collagen type IV.

DISCUSSION

Perlecan is known to be one of the main components of the ECM. We recently showed that its expression decreases substantially during terminal differentiation of skeletal muscle [Larrain et al., 1997a]. The present investigation has determined that perlecan expressed on the myoblast surface can be solubilized using a nonionic detergent such as Triton X-100. A maximal effect was observed at a concentration of 0.05%, which in addition released about 80% of total cytosolic LDH. By contrast, neither RGD peptides, heparin nor high salt, used alone or in combination, were able to displace perlecan located on myoblast surface. These results are in agreement with previous findings in human carcinoma cells [Iozzo, 1984], in which perlecan could only be displaced from the cell surface by detergent, and not high concentrations of heparin. We interpretate our results as being indicative that perlecan is interacting with the plasma membrane, probably via an unknown receptor that does not require an RGD sequence. In this context, it has been shown that aortic endothelial cells synthesize and deposit perlecan, which interacts via its protein core with β1 and β3

Fig. 2. Perlecan is solubilized from the myoblast cell surface by Triton X-100. Myoblasts were grown on coverslips for 3 days and incubated with increasing concentrations of Triton X-100. Cells were then fixed and processed for indirect immunofluorescence as in Fig. 1. **A,F:** Control cells. **B,G:** 0.01% Triton X-100. **C,H:** 0.05% Triton X-100. **D,I:** 0.1% Triton X-100. **E,J:** 0.5% Triton X-100. A–E: Perlecan immunofluorescent staining. F–J: Phase-contrast images. Scale bar = 15 μm.

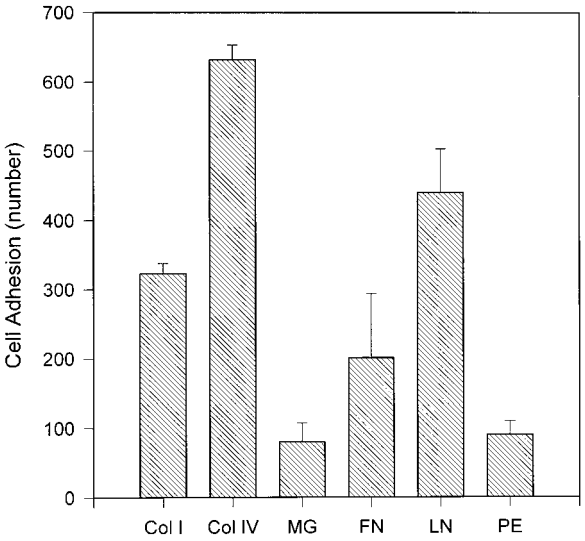

Fig. 5. Attachment of myoblasts to different ECM substrates. Cells prelabeled with [^{35}S]-methionine were seeded in 24-well plates coated with either collagen type I (Col I), collagen type IV (Col IV), matrigel (MG), fibronectin (FN), laminin (LN), or perlecan (PE) and incubated for 10 min. The number of attached cells was determined as explained under Materials and Methods. Values correspond to the mean and standard deviation of four independent experiments.

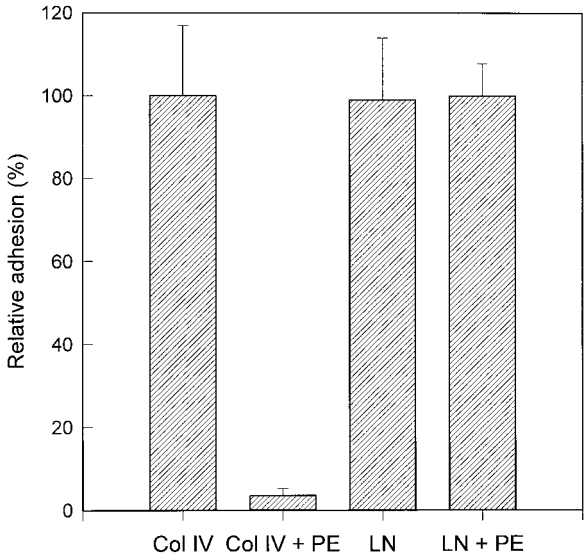

Fig. 6. Attachment of myoblasts to collagen type IV is inhibited by perlecan. Cells were seeded in 24-well plates coated with either collagen type IV (Col IV) or laminin (LN) and then incubated in the presence or absence of perlecan (PE). The attachment of myoblasts to collagen type IV was inhibited almost completely when this substrata was pre-incubated with perlecan, though no effect was observed for laminin. Results correspond to the mean and standard deviation of three independent experiments.

integrins, and this binding was shown to be only partially RGD-dependent [Hayashi et al., 1992]. Furthermore, it has been clearly shown that several tumor cells attach and spread on a perlecan substratum through interactions mediated by β1 integrin [Battaglia et al., 1992].

In order to characterize the biochemical properties of perlecan present on the myoblast surface, cells were extracted with Triton X-100 and radiolabeled proteoglycans isolated through DEAE-Sephacel chromatography, followed by size fractionation through a Sepharose CL-4B column. We found that perlecan synthesized by myoblasts presented a polydisperse profile and eluted with a K_{av} value of 0.45, as determined by inmunological analyses using specific antibodies against murine perlecan. Similar characteristics have been found for perlecan isolated from other sources [Edge and Spiro, 1987; Klein et al., 1988]. This polydisperse size distribution probably reflects a range of perlecan core proteins of varying sizes that undergo specific proteolytic processing as demonstrated previously [Klein et al., 1988; Mohan and Spiro, 1991].

Our findings indicate that perlecan likely participates in myoblast attachment through an interaction with collagen type IV. Attachment of myoblasts to different ECM molecules was tested and cells attached most efficiently to collagen type IV. The attachment of myoblasts to this ECM components was almost completely abrogated by pre-incubation of collagen type IV with exogenous perlecan, suggesting that endogenous cell surface perlecan was involved in this association. No such inhibitory effect was observed when laminin was pre-incubated with perlecan, indicating the interaction to be specific for collagen type IV. Moreover, the attachment of myoblasts to collagen type IV seemed to be mediated through the core protein of perlecan, as both intact perlecan and deglycosylated perlecan inhibited cell attachment to the same extent. In the same way, treatment of myoblasts with antibodies against perlecan prior to their exposure to collagen type IV coated dishes, also inhibited cell adhesion substantially. Overall, our findings suggest that perlecan is present on the cell surface of myoblasts and participates in the adhesion of myoblasts to collagen type IV.

There are several possible functions for the presence of this heparan sulfate proteoglycan in undifferentiated myoblasts. One is to interact with ECM constituents in order to contribute to form a well organized basement membrane, critical for the skeletal muscle contraction of myotubes. Another possible function involves

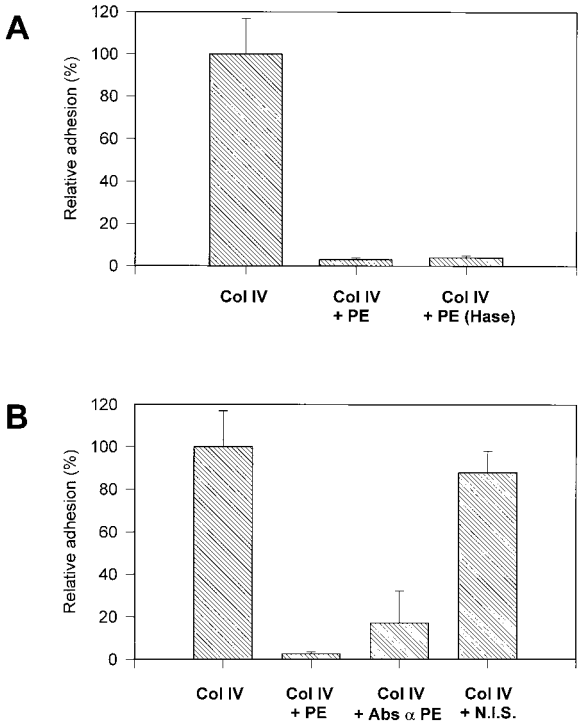

Fig. 7. **A:** The attachment of myoblasts to collagen type IV is inhibited by the perlecan core protein. Cells were seeded in 24-well plates coated with collagen type IV (Col IV) and incubated in the presence of either intact perlecan (PE), or perlecan previously treated with heparitinase (PE+Hase). Attached cells were determined as explained under Materials and Methods. The results correspond to the mean and standard deviation of three experiments. **B:** Antibodies against perlecan inhibit the attachment of myoblasts to collagen type IV. Cells were pre-incubated either with antibodies against perlecan (Abs α PE) or with nonimmune serum (N.I.S.). Cells were then seeded in 24-well plates coated with collagen type IV (Col IV). The attachment of myoblasts to collagen type IV in the presence of perlecan is shown for comparison. The results correspond to the mean and standard deviation of three independent experiments.

its interaction with collagen type IV, shown in this work, which could be important during the development of skeletal muscle. Skeletal muscle cells migrate from somites to hind limb where they form multinucleated fibers [Cossu et al., 1996]. The interaction of perlecan with collagen type IV in this process could be critical for determining the migratory route of myoblasts. An alternative function for perlecan present on the myoblast surface could be an interaction with the basal lamina during the regeneration process induced after injury. Under these circumstances, satellite myoblasts resting under the basal lamina are activated into a proliferative state after which they differentiate and either fuse with pre-existing skeletal fibers or form a new ones [Schultz and McCormick, 1994]. Finally, heparan sulfate proteoglycans are essential for the presentation of growth factors, such as FGF-2 and hepatocyte growth factor/scatter factor, to their transducing receptors [Bernfield et al., 1992; Carey, 1997]. It can be speculated that perlecan might be required on the surface of myoblasts to act as a growth factor co-receptor and allow the presentation and subsequent binding of the growth factor to its signalling receptor [Aviezer et al., 1994a,b]. This possibility is supported by the finding that stable expression of perlecan anti-sense cDNA produced a suppression in the autocrine and paracrine functions of FGF-2 in fibroblasts [Aviezer et al., 1997].

Although the precise function(s) for perlecan on the myoblasts cell surface have yet to be determined, an interaction with collagen type IV, as shown in this paper, seems to be relevant in myoblasts adhesion. Experiments designed to abolish the expression of perlecan in myoblasts are in progress. It is hoped that they will yield valuable information regarding the function(s) of this heparan sulfate proteoglycan on the surface of myoblasts.

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