

Heparin and Related Glycosaminoglycans Modulate the Secretory Phenotype of Vascular Smooth Muscle Cells

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ABSTRACT Previous studies have established a role for heparin-like molecules in the regulation of vascular smooth muscle cell growth and migration *in vitro*. We present data indicating that the secretory phenotype of cultured rat aortic smooth muscle cells can be modulated by exogenous soluble heparin, heparan sulfate, and dermatan sulfate glycosaminoglycans. In the presence of these molecules, smooth muscle cells secrete increased amounts of two noncollagenous proteins (M_r 37,000 and 39,000). This effect can be mimicked by iota carrageenan and dextran sulfate but not by hyaluronic acid, chondroitin-4-sulfate, or chondroitin-6-sulfate. The inductive effect of heparin was dose-dependent and occurred rapidly (within 1 h) with maximal induction (three- to fivefold over controls) occurring after 10–12 h of treatment. The effect was rapidly reversible (within 1 h) and was not altered in the presence of actinomycin D, suggesting regulation at a posttranscriptional level. These data indicate that the biosynthetic expression of specific smooth muscle cell proteins may be determined, at least in part, by components of the smooth muscle cell extracellular matrix.

Several biological activities of vascular smooth muscle cells (SMC)¹ appear to be regulated by heparin and related glycosaminoglycans. Heparin has been shown to inhibit SMC migration after the wounding of cell layers *in vitro* (18), to inhibit SMC proliferation *in vitro* (4, 13) and *in vivo* after arterial injury (8), and to inhibit SMC “modulation” from a contractile to a synthetic/growth factor-responsive phenotype (7).

The importance of these findings is supported by indications that inhibitory heparin-like molecules may normally be present in the quiescent vascular wall. Early studies (11) demonstrated the production of heparin-like glycosaminoglycans by the vascular endothelium *in vitro*. More recent studies have indicated that heparan sulfates derived from quiescent cultures of aortic endothelium (5) and from confluent cultures of vascular smooth muscle cells (10) are potent inhibitors of SMC growth. A heparinase-degradable inhibitory activity was found to be released from endothelial cell cultures by the action of a specific endoglycosidase (5) present in platelets (19) and in SMC (10). This enzyme liberates small heparan sulfate glycosaminoglycans, 8–12 saccharides in length, by cleaving larger chains at glucuronyl-glucosamine linkages (19). The presence of this enzyme in the vessel wall, and the implied generation of active heparan sulfates in the SMC

matrix, have led to the development of a model for vascular SMC growth control in which matrix heparin-like glycosaminoglycans act as endogenous chalones (5).

The mechanism(s) by which heparin inhibits SMC growth, migration, and “modulation” are not known. It is likely that heparin may elicit a pleiotropic response in vascular SMC; in the presence of heparan sulfate molecules in the SMC matrix, many functions associated with the differentiation of these cells may be affected. We have begun to analyze the role of these matrix molecules in the regulation of SMC protein synthesis and gene expression. We have found that heparin-treated rat SMC produce markedly increased amounts of a cell layer-associated collagenous protein with a molecular weight of 60,000 (17). In this paper we describe changes in the pattern of secreted proteins after treatment of rat SMC with exogenous glycosaminoglycans, emphasizing the coordinately induced secretion of two noncollagenous polypeptides (M_r 37,000 and 39,000). Our observations suggest that the biosynthetic phenotype of vascular SMC may be determined, at least in part, by the character of the SMC extracellular matrix.

MATERIALS AND METHODS

Cell Culture: Rat SMC were grown from aortic explants essentially as described previously (18). Cultures were maintained at 37°C in a humidified 5% CO₂ atmosphere in Waymouth's medium (Rehatuin, Reheis Chemical Co.,

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¹ Abbreviation used in this paper: SMC, smooth muscle cells.

Kankakee, IL) supplemented with 10% fetal calf serum (HyClone, Sterile Systems, Inc., Logan, UT), 100 U/ml penicillin, 0.1 mg/ml streptomycin, and 0.4 $\mu\text{g/ml}$ *N*-butyl-*p*-hydroxybenzoate. SMC were subcultured at a 1:4 split ratio; cells in the first through fifth passage were plated into 20- or 35-mm wells for metabolic labeling. Except where indicated, only multi-layered, overconfluent SMC cultures were used in experiments. Under these conditions, SMC migration and proliferation were at minimal levels.

Glycosaminoglycan Treatment and Metabolic Labeling: SMC cultures were exposed to soluble glycosaminoglycans (usually at 100 $\mu\text{g/ml}$) added to stock culture medium that contained 10% serum for indicated periods of time. No detectable morphological alterations followed treatment of SMC with glycosaminoglycans. Heparin (type I, 167 United States Pharmacopeia U/mg), hyaluronic acid and dextran sulfates were obtained from Sigma Chemical Co., St. Louis, MO. Low molecular weight non-anticoagulant heparin (RD heparin) was provided by Hepar Industries, Franklin, OH. Iota carrageenan was a gift of Dr. T. Wight, University of Washington. Heparin sulfate (from bovine kidney), chondroitin-4-sulfate, chondroitin-6-sulfate, and dermatan sulfate were obtained from Miles Laboratories, Inc., Elkhart, IN. After exposure of SMC to glycosaminoglycans, cells were metabolically labeled with 50 $\mu\text{Ci/ml}$ of [2,3- ^3H]proline (Amersham Corp., Arlington Heights, IL; 33 Ci/mmol), 40–60 $\mu\text{Ci/ml}$ [^{35}S]methionine (New England Nuclear, Boston, MA; 1166.5 Ci/mmol), or 50 $\mu\text{Ci/ml}$ [5,6- ^3H]uridine (Amersham Corp., 46 Ci/mmol) in Waymouth's medium lacking proline or methionine as required. All labeling media were supplemented with 50 $\mu\text{g/ml}$ sodium ascorbate, 80 $\mu\text{g/ml}$ β -aminopropionitrile fumarate, antibiotics, 0.1 mg/ml bovine serum albumin, and glycosaminoglycans as indicated. Cells were labeled for 1–24 h at 37°C. Actinomycin D (Sigma Chemical Co.) was used at 2.5–5 $\mu\text{g/ml}$. The efficacy of actinomycin treatment was determined by incorporation of [^3H]uridine into RNA.

Sample Preparation and SDS PAGE: We harvested metabolically labeled culture medium into protease inhibitors at 4°C, producing a final concentration of 0.2 mM phenylmethanesulfonyl fluoride, 10 mM *N*-ethylmaleimide, 2.5 mM EDTA, and 3 $\mu\text{g/ml}$ pepstatin. Proteins were then precipitated with 10% trichloroacetic acid. Radio-labeled secreted proteins were resolved by SDS PAGE according to Laemmli (14), using 8 or 10% separating gels that contained urea, and 3% stacking gels. Gels were processed for fluorescence autoradiography by permeation with dimethyl sulfoxide and 2,5-diphenyloxazole (3). Gels were then dried and exposed to x-ray film (Kodak X-Omat) at -70°C . All gels presented herein were run under reducing conditions (2 mM dithiothreitol). Quantitation of radioactivity incorporated into specific bands was achieved by scanning underexposed gels (to insure linear conditions) with a Helena Quick-Scan gel scanner (Helena Laboratories, Beaumont, TX).

RESULTS

Overview of Heparin-induced Changes in SMC Secretory Phenotype

When rat SMC were treated for 72 h with 100 $\mu\text{g/ml}$ heparin preceding a 24 h metabolic labeling with [^3H]proline, several reproducible changes were observed in the pattern of proteins resolved by SDS PAGE. We have previously reported that heparin-treated SMC cell layers contain markedly increased amounts of a 60,000-mol-wt collagenous protein (17). Analysis of proteins secreted into the culture medium revealed reduced secretion by heparin-treated cells of type III relative to type I procollagen (Fig. 1, left panel). This observation will be described in greater detail at a later time.

Heparin-treated SMC secreted increased amounts of two polypeptide chains that were not detectable or barely detectable in [^3H]proline-labeled control culture medium (Fig. 1, right panel). Under reducing conditions, these chains migrated on SDS PAGE with apparent molecular weights of 37,000 and 39,000, according to globular protein standards. Data presented in this paper describe the kinetics and characteristics of the induction of these proteins by heparin.

When SMC proteins were metabolically labeled with [^{35}S]methionine or [^{35}S]cysteine, the M_r 37,000 and 39,000 proteins were consistently detected, in low amounts, in control

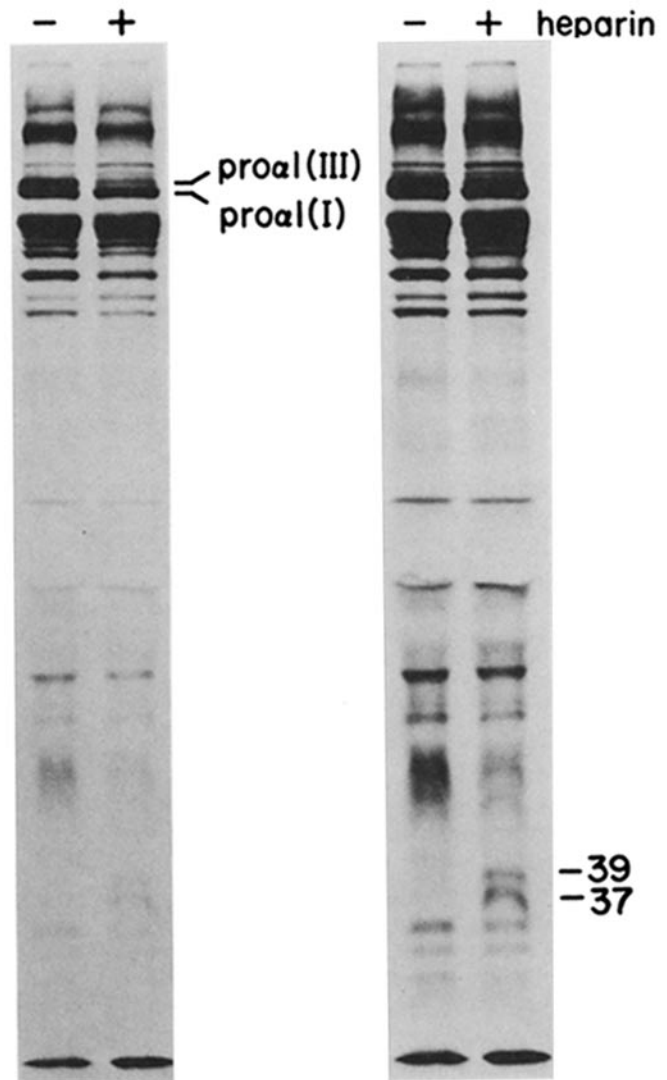


FIGURE 1 Overview of heparin-induced changes in SMC secretory phenotype. Rat SMC were cultured to overconfluence in 20-mm multiwell plates and then treated for 72 h in the presence of 10% fetal calf serum, with or without 100 $\mu\text{g/ml}$ heparin. The cultures were subsequently labeled for 24 h with [^3H]proline, in the presence or absence of heparin. Medium proteins were harvested into protease inhibitors and resolved by SDS PAGE (8% separating gel) followed by fluorography. Both panels represent autoradiographs of the same gel, exposed to different densities for clarity of presentation. In the left panel (*left lane*), the relative densities of the *proα1(I)* collagen band (identified by immunoprecipitation) and the *proα1(III)* collagen band are similar. In the presence of heparin (*right lane*), the *proα1(III)* band is significantly less intense. The right panel illustrates the induced secretion, in the presence of heparin, of two proteins with M_r 37,000 and 39,000.

culture media (see Fig. 4). In addition, another heparin-related change in phenotype was observed. In the presence of heparin, SMC released increased amounts of a protein with a reduced M_r of $\sim 180,000$; this protein has been identified by immunoprecipitation as thrombospondin. This effect (see Fig. 6) is currently under investigation. Heparin-induced changes in secretory phenotype were not observed in cultured human fetal foreskin fibroblasts or in bovine aortic endothelial cells.

Nature of the 37,000- and 39,000-mol-wt Proteins

At present, the nature of the M_r 37,000 and 39,000 proteins, and the relationship between the two, are not clear. Neither protein was degraded when SMC medium was incubated with purified bacterial collagenase under conditions that permitted the selective digestion of known collagens (data not shown). Cross-incubation studies were performed to assess the possibility that the 37,000- and 39,000-mol-wt proteins were produced by the action of heparin on a heparin-inducible or heparin-activated protease leading to cleavage of larger cellular products. Control SMC were labeled with [35 S]methionine and the media harvested, dialyzed to remove free methionine, lyophilized, redissolved in Waymouth's medium, and incubated with control or heparin-stimulated (24 h) SMC cultures for 2 h. No detectable [35 S]methionine-labeled 37,000- and 39,000-mol-wt proteins were produced by the incubation of control, prelabeled medium proteins with heparin-induced cells. It is also possible that the 37,000- and 39,000-mol-wt proteins are produced by heparin-activated proteolytic degradation of a higher molecular weight, cell layer-associated protein. However, no change in cell layer proteins consistent with such an effect can be detected by SDS PAGE (see Fig. 5).

Glycosaminoglycan Specificity of Induction of the 37,000- and 39,000-mol-wt Proteins

To determine the specificity of the SMC response to various polyanions, we treated SMC cultures with 100 μ g/ml of soluble glycosaminoglycans for 72 h before metabolic labeling. Cultures were labeled with [3 H]proline for 24 h in the presence of the appropriate glycosaminoglycan. Control cells secreted little or undetectable amounts of the M_r 37,000 and 39,000 proteins. These molecules were produced in significant amounts in the presence of heparin, heparan sulfate, and dermatan sulfate (Fig. 2), but not in the presence of other glycosaminoglycans (hyaluronic acid, chondroitin-4-sulfate, and chondroitin-6-sulfate). The inductive effect of heparin could be mimicked by low and high molecular weight forms of dextran sulfate and by iota carrageenan, a highly sulfated polyanion found in *Gigartinales* red algae. Low molecular weight RD heparin (Hepar, 67 United States Pharmacopeia U/mg) was also effective in inducing the production of the M_r 37,000 and 39,000 proteins, indicating that molecular determinants of the glycosaminoglycans, rather than anticoagulant activity, are responsible for the effects shown. The purity of our heparin, heparan sulfate, and dermatan sulfate preparations was ascertained by paper chromatography on cellulose acetate using a cadmium acetate buffer. Heparin and heparan sulfate migrated as distinct, separate bands; the dermatan sulfate migrated as one major band with a minor, more highly charged "shoulder." Studies to determine the active moieties in these preparations are now on-going.

Dose-Dependence of Induction of the 37,000- and 39,000-mol-wt Proteins by Heparin

Next, we examined the dose-dependence of induction of the 37,000- and 39,000-mol-wt proteins. SMC were treated with heparin, at increasing concentrations (0.01–1,000 μ g/ml), during a 2-h labeling period with [35 S]methionine. Heparin was effective in stimulating increased production of the

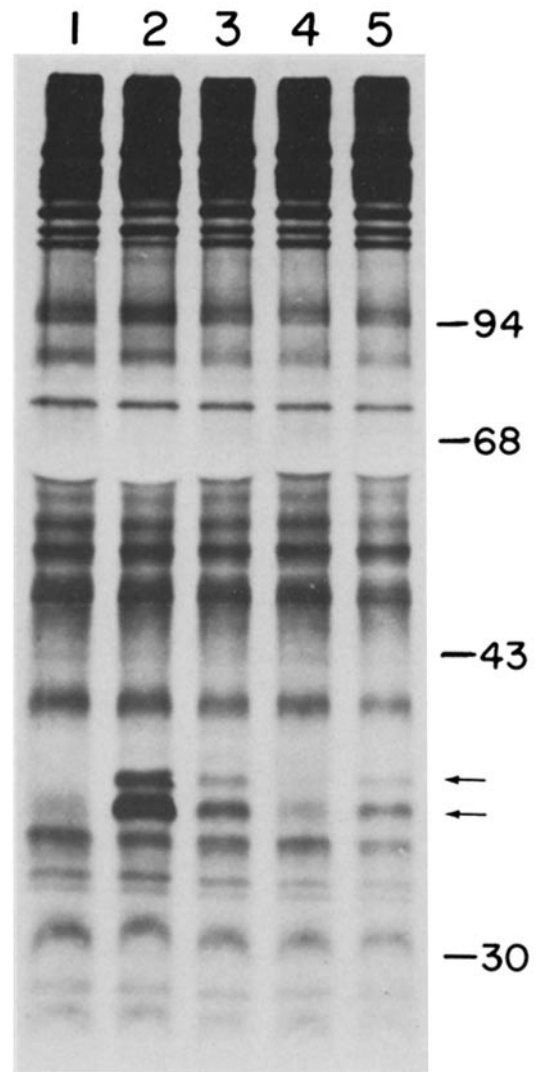


FIGURE 2 Glycosaminoglycan specificity of induction of the 37,000- and 39,000-mol-wt proteins. SMC cultures were treated, as described in Fig. 1, with 100 μ g/ml of various glycosaminoglycans. [3 H]Proline-labeled secreted cell products were subsequently analyzed by SDS PAGE (10% separating gel) and fluorography. Products from control SMC (lane 1) and from SMC treated with heparin (lane 2), heparan sulfate (lane 3), chondroitin-4-sulfate (lane 4), and dermatan sulfate (lane 5) are shown. Note the induction of 37,000- and 39,000-mol-wt proteins by heparin and, to a lesser extent, by heparan sulfate and dermatan sulfate. The effects of other glycosaminoglycans tested are given in the text. The position of migration of molecular weight standards ($\times 10^{-3}$) are shown to the right. Arrows indicate positions of the M_r 37,000 and 39,000 proteins.

37,000- and 39,000-mol-wt proteins at concentrations as low as 0.01 μ g/ml, with a maximal effect near 100 μ g/ml (Fig. 3). It should be emphasized that commercially prepared heparin may contain only a small percentage of the moiety active in inducing the synthesis of the M_r 37,000 and 39,000 proteins. This point has been emphasized by Fritze and Rosenberg (10), who reported that SMC-derived heparan sulfates were 40-fold more active in inhibiting the growth of cultured SMC than was commercial heparin. Purification of the active moiety from heparin preparations (in progress) should result in a marked shift in the dose-response curve.

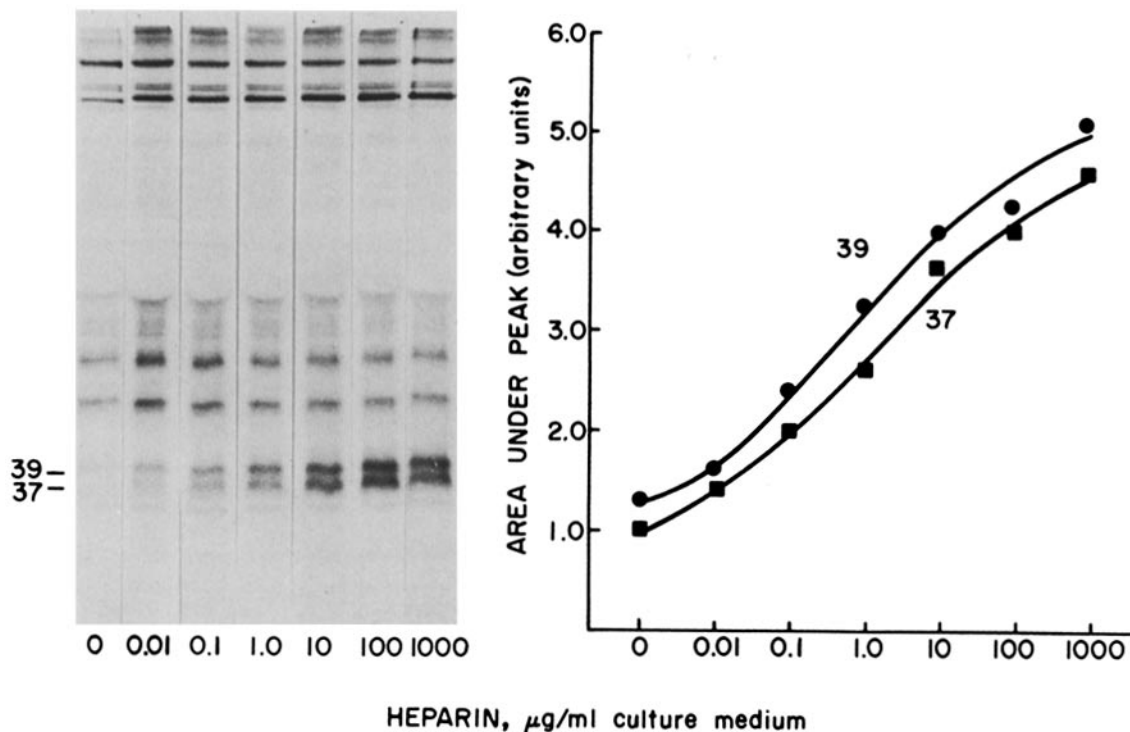


FIGURE 3 Dose-dependency of induction of the 37,000- and 39,000-mol-wt proteins. SMC cultures were labeled with [³⁵S]-methionine for 2 h in the presence of increasing amounts of heparin (concentrations indicated on abscissa). Medium proteins were resolved by SDS PAGE (10% separating gel) followed by fluorography (left). Quantitation of the relative densities of the 37,000- and 39,000-mol-wt protein bands is presented at right. Note that the proteins are significantly induced at heparin concentrations >0.1 µg/ml.

Time Course of Induction of the 37,000- and 39,000-mol-wt Proteins by Heparin

The time course of action of heparin on induction of the 37,000- and 39,000-mol-wt proteins was determined by incubating SMC cultures with 100 µg/ml heparin from 0–22 h, then metabolically labeling the cells with [³⁵S]methionine for 2 h in the absence or presence of heparin. As presented in Fig. 4, secretion of the *M_r* 37,000 and 39,000 proteins was coordinately induced within 2 h of heparin treatment. Other experiments (not shown) have demonstrated induction of these molecules within 1 h of heparin addition. In the continued presence of heparin, 37,000- and 39,000-mol-wt protein synthesis increased to maximally induced levels of up to fivefold over control values. In some experiments, induction of both proteins occurred to a similar extent (see Figs. 1, 3, 6, and 7); in most experiments the 37,000-mol-wt protein was induced to a greater extent than was the 39,000-mol-wt protein (see Figs. 2, 4, and 5). Maximal induction was achieved after 10–12 h of heparin treatment.

Analysis of Possible Heparin-mediated Release of the 37,000- and 39,000-mol-wt Proteins from the Cell Layer Compartment

Our time course experiments indicated that heparin-treated SMC secrete increased amounts of 37,000- and 39,000-mol-wt proteins within 1 h of heparin addition. We examined the possibility that this rapid secretion was effected by release of these molecules from either the extracellular matrix or from an intracellular pool of normally synthesized but not secreted proteins. As shown in Fig. 5 (lanes 1 and 2), no change in cell

layer proteins could be detected after metabolic labeling with [³⁵S]methionine for 2 h in the presence of heparin, suggesting that the 37,000- and 39,000-mol-wt polypeptides are not major cell layer proteins that are displaced by heparin. This conclusion is supported by experiments in which we analyzed the ability of heparin to release prelabeled 37,000- and 39,000-mol-wt proteins into the culture medium. SMC were pre-labeled with [³⁵S]methionine for 2 h in the absence of heparin. The label was then chased with or without heparin in medium that contained a 10-fold excess of nonradioactive methionine; after 4 h, the chase medium was collected and analyzed. During the 4 h-chase period, SMC released <10% of the amount of acid-precipitable radioactivity released by the cells during the 2-h pulse period. Consequently, the fraction of medium proteins required to provide equal numbers of counts in lanes 5 and 6 in Fig. 5 was considerably greater than that required for lanes 3 and 4. The small increase in the density of the 37,000-mol-wt protein band observed when heparin was present during the chase period (Fig. 5, lane 6) can be attributed to an induction of this protein in the presence of residual-free methionine. These data indicate that heparin treatment of SMC does not induce significant secretion or release of these molecules from stable, constantly synthesized, cellular storage pools.

However, it is possible that, before secretion, SMC continually synthesize and rapidly degrade the 37,000- and 39,000-mol-wt proteins. In this case, one could hypothesize that heparin induces SMC to shunt specific intracellular vesicles to the plasma membrane rather than to lysosomes. It is also conceivable that heparin may inhibit the normal incorporation of nascent 37,000- and 39,000-mol-wt proteins into the cell layer compartment, but is unable to effect release of these

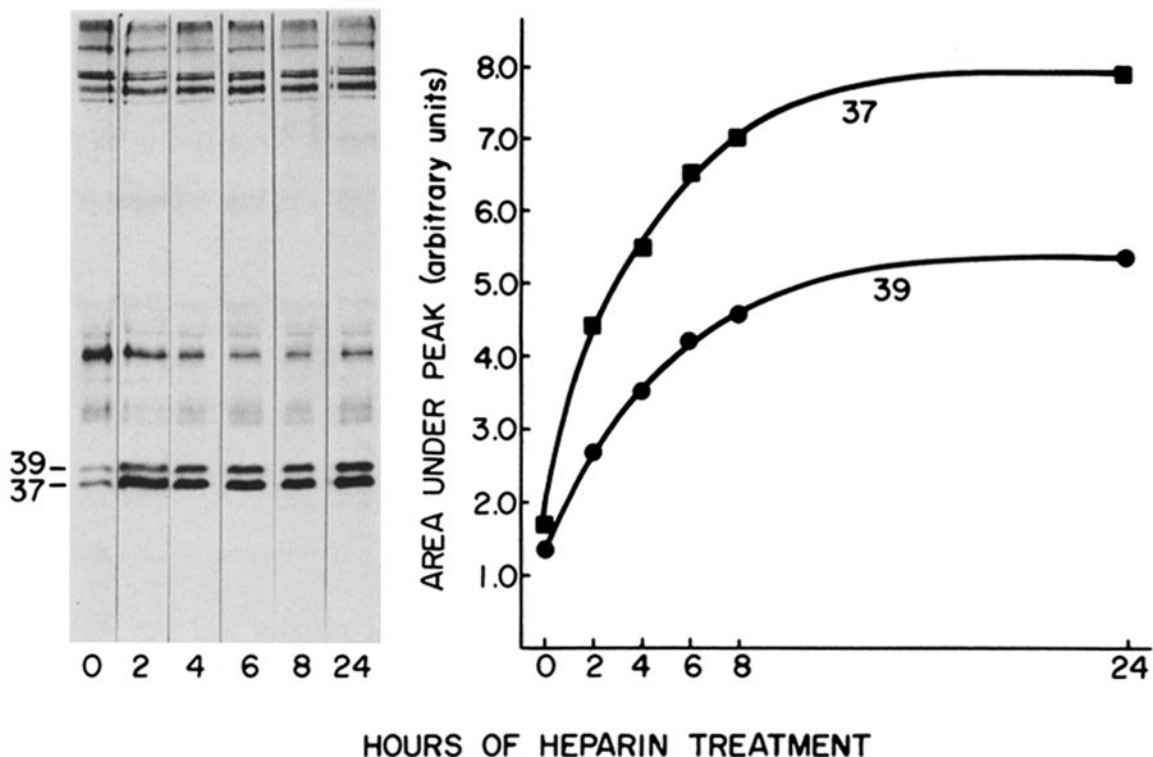


FIGURE 4 Time course of induction of the 37,000- and 39,000-mol-wt proteins by heparin. SMC cultures were treated with 100 $\mu\text{g/ml}$ heparin for the times indicated. For each time point, cells were labeled with [^{35}S]methionine for the final 2 h of each treatment period. Medium proteins were resolved by SDS PAGE (10% separating gel) followed by fluorography (left). Quantitation of the relative densities of the 37,000- and 39,000-mol-wt protein bands is presented at right. Note that induction of both proteins occurs within 2 h and reaches maximal levels after 10–12 h of heparin treatment.

molecules subsequent to their deposition. While we cannot conclusively eliminate these possibilities, we feel that the available data support our conclusion that the increased appearance of the 37,000- and 39,000-mol-wt polypeptides in the culture medium results from enhanced synthesis of these proteins.

Rapidity of Deinduction of the 37,000- and 39,000-mol-wt Proteins after Heparin Removal

To determine the duration of the heparin effect, SMC were treated with heparin (100 $\mu\text{g/ml}$) for 2 h; the cells were then washed and re-fed fresh control medium for 1 or 3 h followed by metabolic labeling with [^{35}S]methionine for 2 h in the absence of heparin. As shown in Fig. 6, the effect of heparin on secretion of 37,000- and 39,000-mol-wt proteins was completely reversible within 1 h after removal of heparin from the cultures. These data indicate the short-lived nature of this SMC response to heparin; cells must be continuously exposed to heparin to maintain a continuously high level of 37,000- and 39,000-mol-wt protein production.

Also shown in Fig. 6 is the effect of heparin on the release (and possibly the synthesis) of a protein with a reduced M_r of 180,000. This protein, identified as thrombospondin, is present in increased amounts in media from cells pre-treated with heparin (lanes 3 and 4).

Actinomycin D Sensitivity of Induction of the 37,000- and 39,000-mol-wt Proteins by Heparin

To determine if the heparin effect on SMC protein induction was mediated at a transcriptional or posttranscriptional

level, we stimulated SMC with heparin for 2 h in the presence or absence of 2.5 or 5 $\mu\text{g/ml}$ actinomycin D, a specific inhibitor of DNA-dependent RNA synthesis. Control experiments indicated that, over a 2.5 h period, total RNA synthesis (expressed as [^3H]uridine incorporation per microgram RNA) was significantly reduced in the presence of 2.5 or 5.0 $\mu\text{g/ml}$ actinomycin D (<30% and <20% of control values, respectively). As shown in Fig. 7, induction of the 37,000- and 39,000-mol-wt proteins by heparin in the first 2 h following heparin treatment was not significantly reduced in the presence of actinomycin D at either concentration. These data indicate that the initial induction of these proteins by heparin is not dependent upon new synthesis of RNA, suggesting that regulation of these proteins by heparin occurs at a posttranscriptional level.

DISCUSSION

In this paper we demonstrate a direct effect of soluble matrix molecules on the expression of specific proteins by rat vascular SMC. In particular, we show that when SMC are cultured in the presence of heparin, heparan sulfate, or dermatan sulfate, the production of two secreted proteins (M_r 37,000 and 39,000) is coordinately induced. Other changes in the biosynthetic phenotype of these cells in response to heparin also occur (17; manuscripts in preparation) and are not detailed here. Cochran et al. (9) have reported that heparin inhibits, in a transitory fashion, the synthesis of a 48,000-mol-wt cell layer protein by rat aortic SMC and stimulates the secretion of a 35,000-mol-wt medium protein. The relationship be-

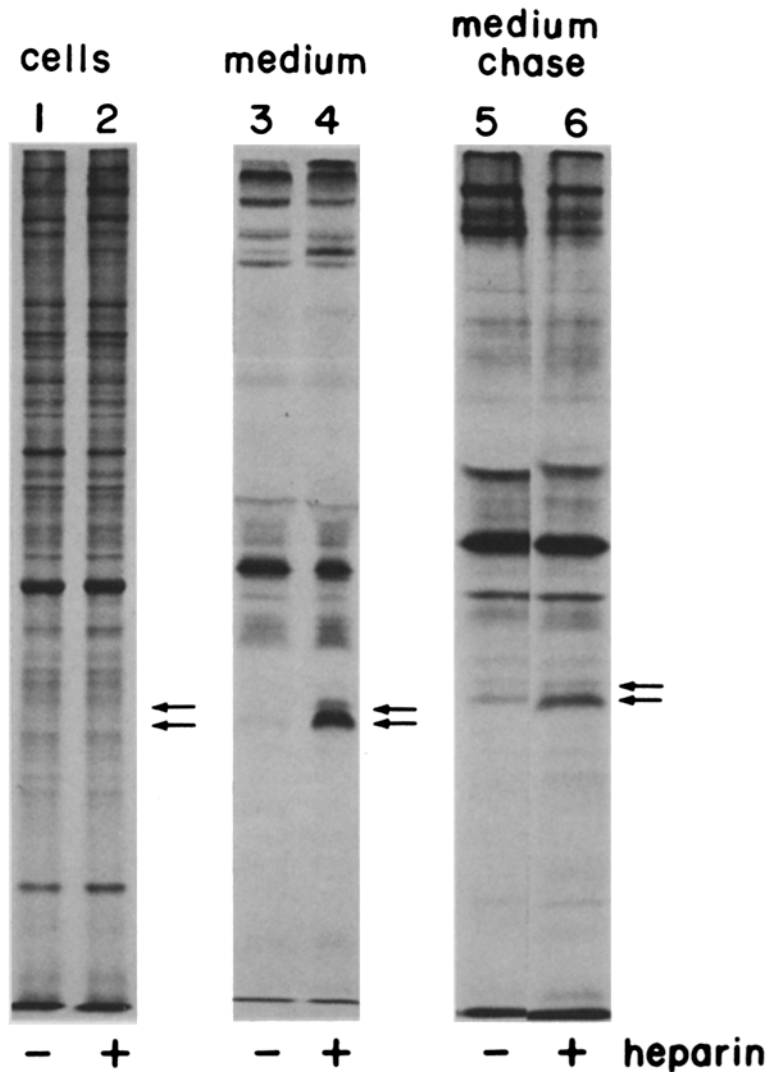


FIGURE 5 Analysis of possible heparin-mediated release of the 37,000- and 39,000-mol-wt proteins from the cell layer compartment. Lanes 1-4: SMC cultures were labeled for 2 h with [³⁵S]methionine in the absence (lanes 1 and 3) or presence (lanes 2 and 4) of 100 μg/ml heparin. Cell layer (lanes 1 and 2) and medium proteins (lanes 3 and 4) were resolved by SDS PAGE (10% separating gels) followed by fluorography. Note that the appearance of the 37,000- and 39,000-mol-wt proteins in the culture medium (lane 4) does not coincide with a detectable change in cell layer proteins (lane 2). Lanes 5 and 6: SMC cultures were metabolically labeled with [³⁵S]methionine for 2 h in the absence of heparin, and then rinsed and incubated with medium unlabeled for 4 h in the absence (lane 5) or presence (lane 6) of heparin. Note that heparin does not significantly increase the amount of pre-labeled 37,000- and 39,000-mol-wt proteins appearing in the culture medium if present only during the 4 h chase period (compare with lanes 3 and 4); equal amounts of radioactivity were loaded onto each lane. These data indicate that heparin does not release significant amounts of these proteins from a stable, preformed cellular or matrix pool. Positions of migration of the 37,000- and 39,000-mol-wt proteins are indicated for each pair of lanes that were run on different gels.

tween this 35,000-mol-wt protein and the 37,000- and 39,000-mol-wt proteins described in this work remains to be established. These observations, together with those data documenting the inhibitory effects of heparin on SMC growth (4, 5, 8, 13) and migration (18), suggest a major role for endogenous heparin-like molecules in determining the functional characteristics of vascular SMC.

Modulation of SMC activity and phenotype may be an important factor in determining, at least in part, the vascular response to atherogenic injury. Such a response might depend on a number of factors, including the presence of stimulatory growth factors, heparin-like inhibitors, and molecules capable of binding (and perhaps inactivating) endogenously produced heparan sulfates (5). Our data suggest that modulation be-

tween control and heparin-induced phenotypes can occur very rapidly; the data for heparin inhibition of migration appear to be similar (18). The relationship between the induction of specific SMC proteins and the inhibition of growth and/or migration by heparin is not yet known. It is possible that the 37,000- and 39,000-mol-wt proteins described herein, the 60,000-mol-wt collagenous cell layer protein (17), or other undetected heparin-inducible proteins may be causally related to these inhibitory activities. We have observed (unpublished observations) that production of the 37,000- and 39,000-mol-wt proteins is not increased in cells growth-arrested for 96 h in plasma-derived serum, indicating that the induction is not a consequence of the inhibition of growth or migration by heparin.

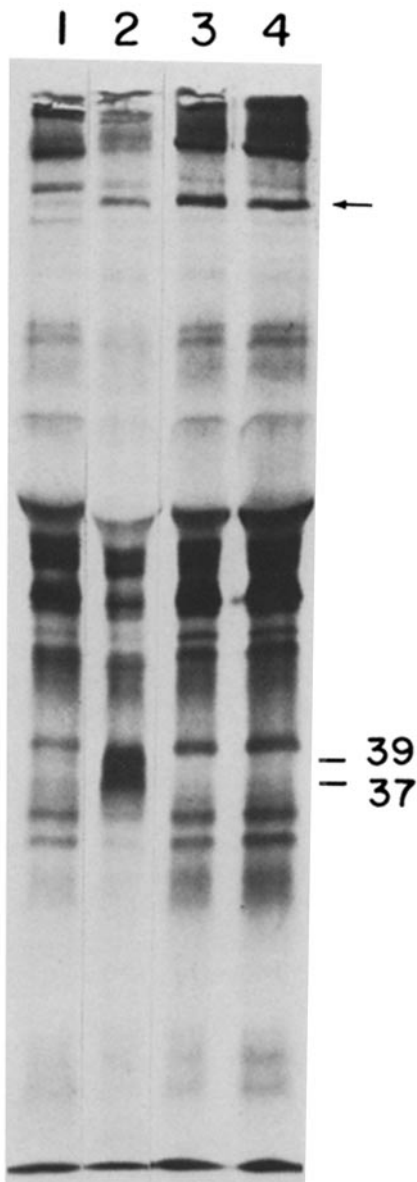


FIGURE 6 Rapidity of deinduction of the 37,000- and 39,000-mol-wt proteins after heparin removal. Proteins resolved in lane 1 represent secreted proteins from control cultures metabolically labeled for 2 h with [³⁵S]methionine. When cultures are labeled for 2 h in the presence of 100 μ g/ml heparin (lane 2), the 37,000- and 39,000-mol-wt proteins are induced. Lanes 3 and 4 illustrate proteins secreted by cells treated with 100 μ g/ml heparin for 2 h, then incubated 1 h (lane 3) or 3 h (lane 4) in the absence of heparin followed by a 2-h metabolic labeling also in the absence of heparin. Note that cells labeled 1–3 h after removal of heparin do not produce appreciable amounts of the 37,000- and 39,000-mol-wt proteins. Arrow indicates the position of thrombospondin (reduced M_r , ~180,000).

The failure of actinomycin D to inhibit the increased synthesis of the M_r 37,000 and 39,000 proteins supports the hypothesis that the expression of these proteins is regulated by heparin at a posttranscriptional level. This conclusion is also supported by the rapid kinetics of induction and deinduction. In other experiments (unpublished observations) we have observed that actinomycin D treatment selectively reduced production of the 37,000- and 39,000-mol-wt proteins

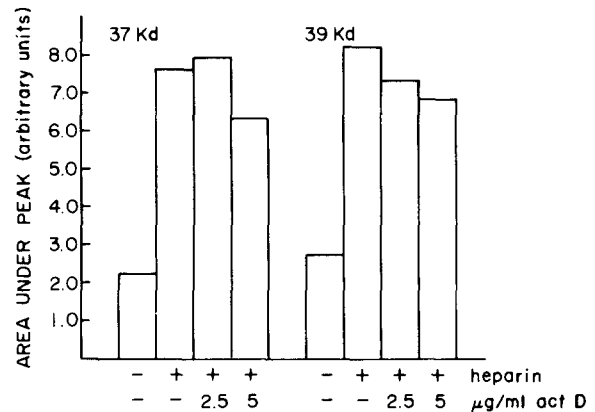


FIGURE 7 Actinomycin sensitivity of induction of the 37,000- and 39,000-mol-wt proteins by heparin. SMC cultures were either not treated or pretreated for 1 h with 2.5 μ g/ml or 5.0 μ g/ml actinomycin D before metabolic labeling with [³⁵S]methionine in the absence or presence of 100 μ g/ml heparin. Medium proteins were then subjected to SDS PAGE, fluorography, and quantitative gel scanning. The relative areas under the 37,000- and 39,000-mol-wt peaks for each experimental condition are presented. Note the failure of actinomycin D treatment to inhibit the inductive effects of heparin at this early time period.

over a 30-h period, suggesting that some form of transcriptional control may be superimposed on the initial translational or posttranscriptional control operative at early time points of induction. Analysis of polysomal and total mRNA from control, 2-h heparin-induced cells, and 24-h heparin-induced cells might clarify the mechanisms of regulation of these proteins.

Our observations are also significant, in a broader sense, in view of current interest concerning the proposed role of the extracellular matrix in the regulation of cellular growth (2, 12), biosynthetic phenotype (15, 16), and gene expression (see reference 1 for review). In our studies, we have added exogenous glycosaminoglycans, in a soluble form, to SMC culture media and have observed very rapid phenotypic modulation. How is this regulation effected? A number of possibilities exist: (a) Heparin may interact with other matrix molecules to effect changes in SMC cell shape by virtue of the close association between the extracellular matrix and the internal cytoskeleton. Such cell shape changes may alter the biosynthetic phenotype through differential association of specific mRNA species with the cytoskeleton (1). Note, however, that the cells used in these experiments are overconfluent, surrounded by a dense matrix, and exhibit no detectable change in morphology in response to heparin (unpublished observations). (b) Heparin may interact with the cell surface via receptors and elicit a "second messenger" response intracellularly. (c) Heparin may be internalized by fluid-phase or receptor-mediated endocytosis and delivered to an intracellular site of action. Evidence for the binding and uptake of heparin by SMC in culture has recently been presented (6); these authors reported that internalization of heparin was rapid (within 15 min) and occurred, at least in part, via a coated vesicle pathway.

The effects of heparin on vascular smooth muscle cells are cell-specific, glycosaminoglycan-specific, and varied in the range of biological responses effected. Heparin-like molecules are now known to regulate the proliferative capacity of SMC

(4, 5, 8, 13), the rate of migration following in vitro experimental wounding (18), the "modulation" of SMC to a growth-factor sensitive phenotype (7), and the SMC biosynthetic phenotype (9, 17; this paper). We therefore envision the effects of heparin on vascular SMC to be pleiotropic in nature, possibly reflecting the characteristics of a separate and specific heparin-induced SMC phenotype.

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