

Amendment history:

- [Correction](#) (January 1982)

Involvement of cell surface heparin sulfate in the binding of lipoprotein lipase to cultured bovine endothelial cells.

K Shimada, ... , W H Douglas, B L Fanburg

J Clin Invest. 1981;[68\(4\)](#):995-1002. <https://doi.org/10.1172/JCI110354>.

Research Article

It has been postulated that lipoprotein lipase, an enzyme important in the uptake of fatty acids into tissues, is bound to the vascular endothelial cell surface and that this binding occurs through attachment to heparinlike glycosaminoglycans. Furthermore, it is thought that heparin releases the enzyme from its attachment to the endothelium into the circulation. These hypotheses have never been tested directly in cell systems in vitro. In the present study we have directly evaluated the interaction of lipoprotein lipase, purified from bovine skim milk with monolayer cultures of endothelial cells, isolated from bovine pulmonary artery. Endothelial cells in primary culture had no intrinsic lipoprotein lipase activity but were able to bind lipoprotein lipase quantitatively. The binding reached equilibrium and was saturable at 0.24 nmol of lipoprotein lipase/mg of cell protein. The concentration of lipoprotein lipase at half-maximal binding was 0.52 microM. Bound lipoprotein lipase could be detached from cultured cells by increasing concentrations of heparin, and at and above 0.6 microgram/ml of heparin, 90% of the cell-bound lipoprotein lipase activity was released. Heparan sulfate and dermatan sulfate released the enzyme to a lesser extent and chondroitin sulfate caused little, if [...]

Find the latest version:

<https://jci.me/110354/pdf>



Involvement of Cell Surface Heparin Sulfate in the Binding of Lipoprotein Lipase to Cultured Bovine Endothelial Cells

KAZUYUKI SHIMADA, PEGGY JO GILL, JEREMIAH E. SILBERT, WILLIAM H. J. DOUGLAS, and BARRY L. FANBURG, *Pulmonary Division, Department of Medicine, and Department of Anatomy and Cellular Biology, Tufts-New England Medical Center, Boston, Massachusetts 02111; Connective Tissue-Aging Research Laboratory, Veterans Administration Outpatient Clinic, and Harvard Medical School, Boston, Massachusetts 02108*

ABSTRACT It has been postulated that lipoprotein lipase, an enzyme important in the uptake of fatty acids into tissues, is bound to the vascular endothelial cell surface and that this binding occurs through attachment to heparinlike glycosaminoglycans. Furthermore, it is thought that heparin releases the enzyme from its attachment to the endothelium into the circulation. These hypotheses have never been tested directly in cell systems *in vitro*. In the present study we have directly evaluated the interaction of lipoprotein lipase, purified from bovine skim milk with monolayer cultures of endothelial cells, isolated from bovine pulmonary artery.

Endothelial cells in primary culture had no intrinsic lipoprotein lipase activity but were able to bind lipoprotein lipase quantitatively. The binding reached equilibrium and was saturable at 0.24 nmol of lipoprotein lipase/mg of cell protein. The concentration of lipoprotein lipase at half-maximal binding was 0.52 μ M. Bound lipoprotein lipase could be detached from cultured cells by increasing concentrations of heparin, and at and above 0.6 μ g/ml of heparin, 90% of the cell-bound lipoprotein lipase activity was released. Heparan sulfate and dermatan sulfate released the enzyme to a lesser extent and chondroitin sulfate caused little, if any, release of lipoprotein lipase. The release of lipoprotein lipase with heparin was

not associated with a release of [35 S]glycosaminoglycans from 35 S-prelabeled cells.

Reductions of lipoprotein lipase binding to endothelial cells and of cell surface-associated [35 S]glycosaminoglycans in 35 S-prelabeled cells occurred in parallel both when cells were pretreated with crude *Flavobacterium heparinum* enzyme before lipoprotein lipase binding and when cells were treated with this enzyme after lipoprotein lipase binding. The removal of heparan sulfate from the cell surface by purified heparinase totally inhibited the binding of lipoprotein lipase by endothelial cells, but the removal of chondroitin sulfate by chondroitin ABC lyase had no effect on this binding.

These results provide direct evidence for lipoprotein lipase attachment to endothelial cells through heparan sulfate on the cell surface, and provide evidence for the release of lipoprotein lipase by heparin through a detachment from this binding site.

INTRODUCTION

Triglycerides, transported in plasma by chylomicrons and very low density lipoproteins, are hydrolyzed by lipoprotein lipase (glycerol ester hydrolase) (EC 3.1.1.3). This hydrolysis is thought to take place at the vascular surface, thereby making fatty acids available for uptake by the tissues (1, 2). Lipoprotein lipase activity is readily released into the circulating blood by heparin injection (3), and the interaction between lipoprotein lipase and heparin *in vitro* has been well studied. Lipoprotein lipase binds to heparin immobilized to agarose gel, and this binding can be dissociated by addition of sodium chloride at high concentration, which suggests that the binding occurs mainly through electrostatic interaction (4, 5). Under

Portions of this work were presented at the Annual Meeting of the American Society for Clinical Investigation, San Francisco, Calif., 25 April 1981.

Dr. Shimada is a recipient of a Parker B. Francis Foundation Fellowship. Address correspondence to Dr. Fanburg, Pulmonary Division, New England Medical Center Hospital, Boston, Mass. 02111.

Received for publication 11 March 1981 and in revised form 4 June 1981.

certain conditions, heparin increases the enzymatic activity and the stability of crude lipoprotein lipase preparations (3, 6), but has no effect on the enzymatic activity of purified lipoprotein lipase (6). Recent studies have demonstrated that lipoprotein lipase also binds to other glycosaminoglycans such as heparan sulfate (a glycosaminoglycan structurally related to heparin), dermatan sulfate, and, to a lesser extent, chondroitin sulfate, although the interaction of lipoprotein lipase with these glycosaminoglycans is weaker than with heparin (7).

Glycosaminoglycans such as heparan sulfate have been reported to be surface components of many mammalian cells in culture (8), including endothelial cells (9–11). Thus, it has been postulated (12, 13) that heparan sulfate on the vascular endothelial cell surface may provide a binding site for lipoprotein lipase. The mechanism of the release of lipoprotein lipase by heparin could then involve a detachment of lipoprotein lipase from its binding site with the formation of a heparin-enzyme complex *in vivo*. The binding of lipoprotein lipase to endothelial cells and a mechanism for release from these cells has not as yet been demonstrated experimentally.

In this report we present data describing the interaction between bovine lipoprotein lipase and cultured calf pulmonary artery endothelial cells. Furthermore, we have related the removal of cell surface glycosaminoglycans to the binding of the lipoprotein lipase. Our data demonstrate that endothelial cells bind lipoprotein lipase and support the concept of the involvement of cell surface heparan sulfate in this binding.

METHODS

Materials. RPMI 1640 medium, antibiotics, and bovine serum were obtained from Flow Laboratories Inc., Rockville, Md. Collagenase (type I, CLS) was purchased from Worthington Biochemical Corp., Freehold, N. J. Hanks' balanced salt solution without phenol red was obtained from Grand Island Biological Co., Grand Island, N. Y. Petri dishes (35 × 10 mm) and 25-cm² flasks were products of Corning Glass Works, Corning, N. Y. Antiserum to human Factor VIII was obtained from Behring Diagnostics, American Hoechst Corp., Somerville, N. J. Cyanogen bromide-activated Sepharose-4B was from Pharmacia Fine Chemicals, Div. of Pharmacia, Inc., Piscataway, N. J. Hydroxylapatite was obtained from Bio-Rad Laboratories, Richmond, Calif. Tri[9,10-³H]oleoylglycerol (470 mCi/mmol) was purchased from Amersham Corp., Arlington Heights, Ill. Sodium [³⁵S]sulfate (840 mCi/mmol) was purchased from New England Nuclear, Boston, Mass. Bovine serum albumin (essentially fatty acid-free), chondroitin 4-sulfate from whale cartilage, chondroitin 6-sulfate from shark cartilage, dermatan sulfate from porcine skin, trypsin, and heparin used for the coupling to Sepharose were obtained from Sigma Chemical Co., St. Louis, Mo. Heparin (165 USP U/mg, from porcine intestinal mucosa) used for binding was obtained from Calbiochem-Behring Corp., American Hoechst Corp., La Jolla, Calif. Bacterial chondroitin ABC lyase was purchased from Miles Labora-

tories, Inc., Elkhart, Ind. Crude *Flavobacterium heparinum* enzyme was prepared as previously described (14). Purified heparinase was supplied by Dr. A. Linker (Salt Lake City, Utah). Heparan sulfate from bovine kidney was provided by Dr. K. Murata (Tokyo, Japan).

Isolation and culture of endothelial cells. Bovine pulmonary artery endothelial cells were isolated from the main-stem pulmonary artery obtained from calves immediately after death, and were cultured as previously described (15) with minor modifications. The artery was removed from the heart and placed in Moscona's saline containing an elevated level of antibiotics (150 U/ml penicillin, 150 µg/ml streptomycin, and 1.25 µg/ml Amphotericin B) and transported at 4°C to the tissue culture laboratory. Each pulmonary artery was drained of excess fluid. One end of the artery was sealed with a hemostat and the vessel was filled with 0.2% collagenase in RPMI 1640 medium containing antibiotics (50 U/ml penicillin, 50 µg/ml streptomycin, and 1.25 µg/ml Amphotericin B) and 1% bovine serum. The free end of the artery was sealed with a hemostat, placed in a sterile beaker containing Moscona's saline and antibiotics, and incubated at 37°C for 25 min. At the end of the incubation period, the cell/enzyme mixture was removed from the lumen of the artery and was transferred to a centrifuge tube containing RPMI 1640 medium supplemented with 10% bovine serum and antibiotics. The cells were sedimented at 160 g for 6 min at 4°C, washed in fresh medium, and placed into 35-mm petri dishes or 25-cm² flasks containing medium as above. These cultures were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air for 4–7 d before use at, or just before, confluence.

Characterization of the primary cultures showed the typical microscopic cobblestone appearance of endothelial cells, contact inhibition in monolayer culture, and failure to form colonies in soft agar. The presence of Factor VIII antigen and angiotensin 1 converting enzyme on the cell surface was demonstrated by indirect immunofluorescence using anti-human Factor VIII (15) or monospecific antibody to rat angiotensin 1 converting enzyme (16).

Purification of bovine milk lipoprotein lipase. Heparin-Sepharose was prepared by the method of Iverius (17). Heparin was coupled to cyanogen bromide-activated Sepharose-4B according to the manufacturer's instructions. Purification of milk lipoprotein lipase was performed by modifications of the method of Iverius and Östlund-Lindqvist (18), as follows: 140 ml of heparin-Sepharose gel was added to 900 ml of bovine skim milk and shaken by inversion on a mechanical shaker for 2 h at 4°C. The gel was washed on a sintered glass filter with 1 liter of 15 mM potassium phosphate buffer (pH 6.8) containing 0.75 M sodium chloride and packed into a column. After washing with 1 bed vol of the same buffer, elution was performed with 1.5 M sodium chloride, 15 mM potassium phosphate buffer (pH 6.8). The enzymatically active fractions of eluates (50 ml) were applied onto a hydroxylapatite column (1.6 × 9 cm) which had been equilibrated with 15 mM potassium phosphate buffer (pH 6.8). The column was washed first with 100 ml of the equilibrating buffer and then with 60 ml of 0.3 M potassium phosphate buffer (pH 6.8) and finally eluted with 0.3 M potassium phosphate buffer (pH 6.8) containing 0.5 M sodium chloride, 20% glycerol. The enzymatically active fractions (50 ml) were pooled and stored at 4°C. The above procedure was repeated on three batches of skim milk. Pooled enzyme fractions (150 ml) were combined and concentrated in an Amicon model 202 cell with a YM-30 membrane to a final volume of 35 ml (Amicon Corp., Scientific Systems Div., Lexington, Mass.). This concentrate was dialyzed against a solution of 3.6 M ammonium sulfate, 15 mM potassium phosphate buffer (pH 6.8). The precipitated protein was collected by centrifugation

and dissolved in 2 ml of 50% glycerol, 15 mM potassium phosphate buffer (pH 6.8), or, in some cases, in 2 ml of Hanks' balanced salt solution containing 2% bovine serum albumin and stored at -20°C . The purified enzyme, with a specific activity of 400–650 μmol of free fatty acid released/min per mg protein in the assay system described below, demonstrated one major band on sodium dodecylsulfate-polyacrylamide gel electrophoresis performed according to the method of Weber and Osborn (19).

Assay for lipoprotein lipase. The assay for lipoprotein lipase activity was performed as previously described by Nilsson-Ehle and Schotz (20), with an emulsion of Tri-[9,10- ^3H]oleoylglycerol as substrate. The radioactive substrate was added so that the specific activity was 800–900 cpm/nmol of triglyceride throughout the experiments. The amount of enzyme assayed was selected so that no more than 7% of the substrate was hydrolyzed during the incubations. Under these conditions the hydrolysis of substrate was linear with time. 1 mU of enzymatic activity was defined as the release of 1 nmol of fatty acid/min at 37°C .

Binding of lipoprotein lipase to endothelial cells. For binding experiments, the purified lipoprotein lipase in glycerol was usually diluted 1,000- to 2,000-fold with RPMI 1640 culture media containing 10% bovine serum. For the study of saturation kinetics, the purified enzyme which had been dissolved in Hanks' balanced salt solution containing 2% bovine serum albumin was used for dilution. 1 ml of medium containing lipoprotein lipase was added to the endothelial monolayer cultures (35-mm petri dishes), which were then incubated for 40 min at room temperature. The culture media were collected and maintained at 4°C before assay for unbound enzymatic activity of lipoprotein lipase. After the cell layer was rinsed twice with 2 ml of iced Hanks' balanced salt solution, 0.5 or 1 ml of this buffer was added and the cells were removed from the petri dishes by scraping with a rubber policeman. Two washes were found to be adequate to remove trace amounts of protein and lipoprotein lipase resulting from contamination of cells by media. The cell suspension was sonicated for 10 s at 4°C . Lipoprotein lipase activities in the incubation media and in the sonicated cell homogenates were determined as described above. Protein was measured according to Lowry et al. (21).

Release of lipoprotein lipase by glycosaminoglycans. After rinsing with Hanks' balanced salt solution as noted above, monolayer endothelial cells with bound lipoprotein lipase were incubated with 0.5 ml of various concentrations of glycosaminoglycans in Hanks' balanced salt solution for 5 min at room temperature. Heparin, chondroitin sulfate (a mixture of equal amounts of chondroitin 4-sulfate and chondroitin 6-sulfate), dermatan sulfate, and heparan sulfate were used. Media with the released lipoprotein lipase were collected and kept at 4°C before assay. After the cell layers were washed, lipoprotein lipase activity remaining on the cells was determined.

Treatment of endothelial cells with glycosaminoglycan-degrading enzymes. The crude *F. heparinum* enzyme preparation contains chondroitin lyase, dermatanase, heparinase, and heparanase (heparitinase) activities, and can therefore degrade all the sulfated glycosaminoglycans produced by the endothelial cells. Enzyme activity was measured by increase in A_{232} due to an unsaturated double bond introduced during cleavage between the hexosamines and uronic acids. This activity was expressed as micromoles of unsaturated double bond appearing from a heparin substrate per hour. Chondroitin ABC lyase activity was monitored at A_{232} with chondroitin sulfate as a substrate, and units were similarly expressed as micromoles per hour. Purified heparinase has no activity on chondroitin sulfate or dermatan sulfate; it degrades

heparin to small sized molecules, and will partially degrade heparan sulfate. Unitage was defined identically to the definition of unitage for the crude *F. heparinum* enzyme.

The crude *F. heparinum* enzyme preparation has no demonstrable protease activity. This has been previously established by incubation of the crude *F. heparinum* preparation with bovine endothelial cell cultures or human skin fibroblast cultures that had been prelabeled with [^3H]leucine or [^3H]proline (14). There was no net release of ^3H -proteins or other ^3H -labeled products under these conditions.

Washed endothelial cell cultures were incubated for 40 min at 37°C in 0.5 ml of Hanks' balanced salt solution with (a) various amounts of crude *F. heparinum* enzyme, (b) 2.4 U/ml of pure heparinase, (c) 60 U/ml of chondroitin ABC lyase, or (d) no added enzyme. After the incubations, cultures were washed, lipoprotein lipase was added, and cell cultures were assayed for lipoprotein lipase binding as described above. In another series of experiments, endothelial cell cultures were incubated with crude *F. heparinum* enzyme for 40 min at 37°C in 0.5 ml of Hanks' balanced salt solution after binding of lipoprotein lipase to the cells. Activities of released and bound lipoprotein lipase were determined.

Release of [^{35}S]glycosaminoglycan from ^{35}S -labeled cells. Endothelial cells were labeled with [^{35}S]sulfate before removal of [^{35}S]glycosaminoglycans with glycosaminoglycan lyases as previously described for cultures of skin fibroblasts (14). To achieve the labeling, culture media containing 25 $\mu\text{Ci/ml}$ of [^{35}S]sulfate were incubated with cells for 24 h in 25- cm^2 culture flasks. After removal of the medium and washing, the cell layers were then incubated for 40 min at 37°C with 1 ml of Hanks' balanced salt solution containing either crude *F. heparinum* enzyme, heparinase, or chondroitin ABC lyase. Each of the solutions was removed to provide an "enzyme" fraction and cultures were then incubated with 0.5 ml of 0.25% trypsin, which lifted all the endothelial cells from the plates. The cell suspensions were centrifuged to obtain a supernate ("trypsinate" fraction) and a cell pellet ("cell" fraction). The trypsinate fraction includes glycosaminoglycans derived from the cell surface and from the solubilized matrix. The cultures were then treated with 0.5 ml of 0.5 M NaOH for the removal of radioactive material that might remain on the plates.

The ^{35}S -labeled products of enzymatic degradation were chromatographed on Whatman No. 1 paper (Whatman, Inc., Clifton, N. J.) with a descending system of butanol/acetic acid/1 M- NH_4OH (2.3:1, vol/vol/vol) (22). Cell and trypsinate fractions were chromatographed on Whatman No. 1 paper in a descending system of 1 M ammonium acetate (pH 7.8)/95% ethanol (2.5 vol/vol). Small amounts of residual [^{35}S]sulfate were easily separated and identified by these techniques. The total ^{35}S -radioactivity exclusive of the free [^{35}S]sulfate in each chromatogram was used to give amounts of ^{35}S -labeled degradation products, [^{35}S]glycosaminoglycans, or [^{35}S]proteoglycans in various fractions.

In addition to the enzymatic degradation experiments, ^{35}S -labeled endothelial cell monolayers were incubated with 1 ml of various concentrations of heparin (0, 0.06, 0.6, 6.0, and 60 $\mu\text{g/ml}$) in Hanks' balanced salt solution for 5 min at room temperature. Media were removed; cells were washed, then incubated with 0.25% trypsin to obtain a media fraction, trypsinate fraction, and cell fraction.

RESULTS

Binding of lipoprotein lipase to endothelial cells. Purified lipoprotein lipase was very unstable, as previously reported (18, 23). The enzyme lost 90%

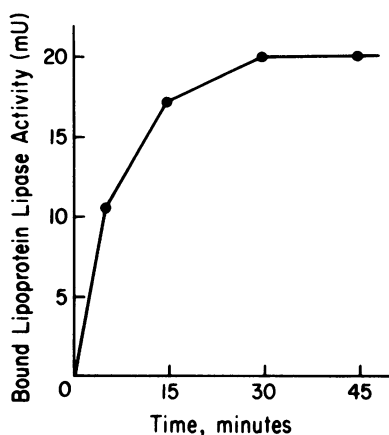


FIGURE 1 Time-course of binding of lipoprotein lipase to endothelial cells. 324 mU of lipoprotein lipase was incubated with endothelial cell cultures in the incubation volume of 1 ml. At various times of incubation, bound lipoprotein lipase activity was determined as described in Methods. The data shown are from one of two experiments with similar results.

of its activity when it was incubated at 37°C for 5 min in Hanks' balanced salt solution. We found, however, that purified lipoprotein lipase activity was stabilized in the presence of endothelial cells or bovine serum, whereas neither substance affected its enzymatic activity. This allowed the quantitative analysis of the lipoprotein lipase binding to endothelial cells in the present system.

Although endothelial cells in culture had no measurable intrinsic lipoprotein lipase activity, the incubation of lipoprotein lipase with monolayer cultures of endothelial cells resulted in binding of lipoprotein lipase to these cells. When 367 ± 13 mU (mean \pm SE of nine separate experiments) of lipoprotein lipase activity was incubated with endothelial cell cultures (0.21 ± 0.02 mg cell protein/dish), as described in Methods, 23 ± 0.8 mU of lipoprotein lipase activity was found in the form associated with cells, and 325 ± 9 mU of lipoprotein lipase activity was recovered as an unbound form in the incubation medium. Thus, there was minimum loss ($\sim 5\%$) of total enzyme activity during incubation.

The time-course for binding of lipoprotein lipase to endothelial cells is shown in Fig. 1. Binding reached a maximum by 30 min at room temperature. Amounts of bound enzymatic activity remained constant thereafter for at least an additional 15 min. Hence, all binding experiments were terminated after 40 min of incubation.

When increasing amounts of lipoprotein lipase were incubated with cells, the amount bound to cells reached a plateau (Fig. 2), which indicates saturation. In experiments carried out with different cell culture preparations, the mean maximal lipoprotein lipase activity

bound to cells was $9,400 \pm 1,900$ mU/mg of cell protein, and the mean concentration of lipoprotein lipase at half-maximal binding (K_m) was $20,200 \pm 1,300$ mU/ml (mean \pm SE of four experiments). These values correspond to 0.24 nmol of lipoprotein lipase/mg of cell protein and a concentration of $0.52 \mu\text{M}$, respectively, on the assumption of a dimeric molecular weight of 100,000, as described by Iverius et al. (18).

Release of lipoprotein lipase by glycosaminoglycans. Fig. 3 demonstrates the release of lipoprotein lipase from cells by increasing concentrations of heparin. The incubation with Hanks' balanced salt solution alone released $\sim 10\%$ of the bound lipoprotein lipase activity. Incubation with $0.6 \mu\text{g/ml}$ heparin resulted in the release of 90% of bound lipoprotein lipase activity and further increase of heparin concentration failed to cause additional lipoprotein lipase release from the cells. Furthermore, Fig. 3 shows that lipoprotein lipase activity released from the cells was quantitatively recovered in the heparin solution without loss or enhancement of lipoprotein lipase activity initially associated with cells.

The lipoprotein lipase releasing effect of heparin was compared with that of other glycosaminoglycans (Table I). Among the glycosaminoglycans tested, heparin displaced the enzyme from cells at the lowest concentration ($0.6 \mu\text{g/ml}$). Heparan sulfate and dermatan sulfate required a higher concentration to displace the enzyme, and chondroitin sulfate failed to release the enzyme even at the highest concentration tested ($30 \mu\text{g/ml}$).

Treatment of endothelial cells with crude F. heparinum enzyme. Effects of cell treatment with crude *F. heparinum* enzyme are shown in Fig. 4. Preincuba-

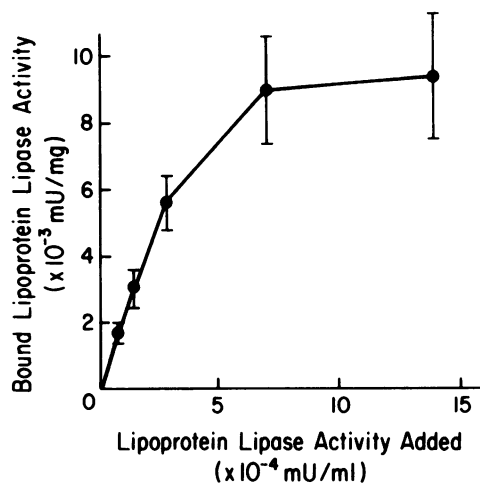


FIGURE 2 Saturability of binding of lipoprotein lipase to endothelial cells. The amount of lipoprotein lipase bound is expressed in mU/mg cell protein as a function of the concentration of lipoprotein lipase in the incubation medium (mU/ml). The mean \pm SE of four separate experiments is shown.

tion of cells with Hanks' balanced salt solution resulted in a decrease of ~25% in lipoprotein lipase binding. When endothelial cells were preincubated with crude *F. heparinum* enzyme at increasing concentrations, they bound progressively smaller amounts of lipoprotein lipase. Endothelial cells incubated with 2.5 U/ml of this enzyme bound only 15% as much lipoprotein lipase activity as untreated cells. The total of bound and unbound lipoprotein lipase activities recovered after incubation with the cells did not differ from that of untreated cells (97–102% of that of untreated cells).

After incubating cell cultures with [³⁵S]sulfate as described in Methods, total [³⁵S]glycosaminoglycan per flask was 6,900±770 cpm (mean±SE of 16 determinations), and the amount of cell surface-associated [³⁵S]glycosaminoglycans (*F. heparinum* enzyme fraction plus trypsinase fraction) accounted for 77±2% of the total [³⁵S]glycosaminoglycans. As shown in Fig. 4, ~25% of the [³⁵S]glycosaminoglycan was removed by incubation with Hanks' balanced salt solution alone for 40 min at 37°C. Increasing amounts of [³⁵S]glycosaminoglycan were removed with increasing amounts of *F. heparinum* enzyme. Only 10% of cell surface-associated glycosaminoglycans initially present was found to remain after the treatment of cells with 2.5 U/ml. The amount of lipoprotein lipase bound to cells treated with *F. heparinum* enzyme was proportional to the amount of cell surface-associated glycosamino-

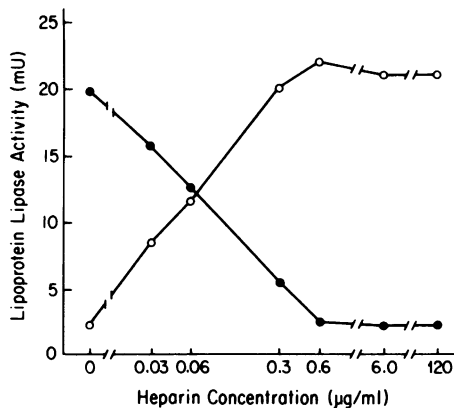


FIGURE 3 Release of cell-bound lipoprotein lipase with heparin. Endothelial cells with bound lipoprotein lipase were incubated with various concentrations of heparin in Hanks' balanced salt solution for 5 min. Cell-bound lipoprotein lipase activity obtained before incubations was 24±5 mU/dish (mean and range of two separate experiments). Lipoprotein lipase activities released into heparin solutions (○) and activities remaining on cells (●) were determined as described in Methods. The mean of two separate experiments is shown. At each concentration of heparin, the observed range of two experiments was <±4%, when the values were expressed as the percentage of the total enzymatic activity recovered after incubation with heparin.

TABLE I
Effects of Various Glycosaminoglycans on the Release of Lipoprotein Lipase Bound to Endothelial Cells

Concentration of glycosaminoglycan µg/ml	Lipoprotein lipase activity released from the cells by:			
	Heparin	Heparan sulfate	Dermatan sulfate	Chondroitin sulfate
0.6	89±3	12*	15*	—‡
3	—‡	36±0.5	44±4	—‡
30	89±1	63±0.5	85±2	9±0

Each value (mean and range of two separate experiments) represents the percentage of the total lipoprotein lipase activity recovered after incubations with glycosaminoglycans. This total activity did not differ significantly from the cell bound enzyme activity obtained before incubations (24±3 mU lipoprotein lipase/dish) for any of the glycosaminoglycans tested.

* Data from only one experiment.

‡ Not tested.

glycans remaining after the identical enzymatic treatment of cells.

In another experiment, treatment with *F. heparinum* enzyme was performed on endothelial cells after their binding of lipoprotein lipase, rather than before lipoprotein lipase binding. Treatment by Hanks' balanced salt solution alone resulted in a small loss of lipoprotein lipase activity bound to cells, but *F. heparinum* enzyme treatment at 0.3 U/ml and 2.5 U/ml reduced

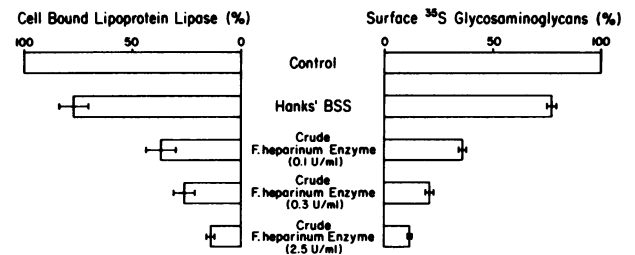


FIGURE 4 Treatment of endothelial cells with crude *F. heparinum* enzyme. (Left) Endothelial cell cultures were treated with various concentrations (0.1, 0.3, and 2.5 U/ml) of crude *F. heparinum* enzyme or Hanks' balanced salt solution (BSS). Lipoprotein lipase activities bound to these cells are expressed as the percentage of the enzyme activity bound to untreated cells (control). The mean of three separate experiments is recorded. Cell-bound lipoprotein lipase activity of control cells was 98±9 mU/mg cell protein (mean±SE of 3 experiments). (Right) Identical enzymatic digestions were performed with [³⁵S]-labeled cell cultures for the study of [³⁵S]glycosaminoglycan release from cells. The amount of cell surface associated [³⁵S]glycosaminoglycan remaining after each enzyme treatment is represented by the [³⁵S]-labeled material in the trypsinase fraction. This is expressed as the percentage of [³⁵S]glycosaminoglycans initially present on the cell surface (enzyme plus trypsinase fractions). Each value represents the mean±SE of four determinations from two separate experiments.

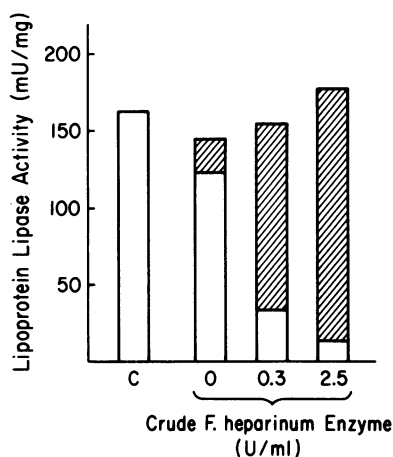


FIGURE 5 Release of cell-bound lipoprotein lipase by treatment of cells with crude *F. heparinum* enzyme. Endothelial cell cultures were incubated with 440 ± 40 mU/ml of lipoprotein lipase (mean and range of two separate experiments) for 40 min at room temperature. Control cultures were determined for cell-bound lipoprotein lipase activity without further treatments (C). Other endothelial cell cultures were then incubated with 0.5 ml of Hanks' balanced salt solution containing crude *F. heparinum* enzyme (0, 0.3, and 2.5 U/ml) for 40 min at 37°C. Lipoprotein lipase activity released into the solution (▨) and that remaining on cells (□) were determined as described in Methods. The data are expressed as the ratio of lipoprotein lipase activity to cell protein for each dish. The mean of two separate experiments is shown. In all cases, the observed range was $< \pm 9\%$ of the mean value of two experiments.

the bound lipoprotein lipase to 21 and 9%, respectively, of that of untreated cells (Fig. 5). Lipoprotein lipase remained active in a cell bound form or in the presence of *F. heparinum* enzyme preparations. Released lipoprotein lipase activity was essentially quantitatively recovered in the solution. Thus, reduction of lipoprotein lipase binding to endothelial cells and removal of cell surface-associated glycosaminoglycans occurred in parallel whether the cells were pretreated with *F. heparinum* enzyme before lipoprotein lipase binding or were treated with this enzyme after lipoprotein lipase binding.

Treatment of cells with purified heparinase and chondroitin ABC lyase. Crude *F. heparinum* enzyme will degrade all the sulfated glycosaminoglycans of endothelial cells. Therefore, experiments were performed with purified heparinase and chondroitin ABC lyase. As shown in Table II, treatment by large amounts of purified heparinase (2.4 U/ml) and chondroitin ABC lyase (60 U/ml) resulted in a total loss of 60 and 65% of the [^{35}S]glycosaminoglycans, respectively. Approximately 25% of the ^{35}S -labeled material could be washed off with buffer alone. Paper chromatograms in the butanol/acetic acid/ammonium system showed that most of the ^{35}S -labeled material released by purified heparinase remained at the origin, which indicates that

it was not extensively degraded, whereas treatment with crude *F. heparinum* enzyme produced a mixture of products that moved from the origin, which indicates substantial degradation. Products of the chondroitin ABC lyase treatments chromatographed as 2-acetamido-2-deoxy-3-O-(β -D-gluc-4-enepyranosyluronic acid)-4-O-sulpho-D-galactose and 2-acetamido-2-deoxy-3-O-(β -D-gluc-4-enepyranosyluronic acid)-6-O-sulpho-D-galactose. These results indicate that heparan sulfate and chondroitin sulfate were selectively removed by treatment with the purified heparinase and chondroitin ABC lyase, respectively.

Identical enzymatic digestions were also performed with unlabeled endothelial cell cultures for the study of lipoprotein lipase binding to cells. Table II shows that endothelial cells treated with purified heparinase bound only 15% as much lipoprotein lipase as untreated cells. On the other hand, cells treated with chondroitin ABC lyase bound as much lipoprotein lipase as those treated with Hanks' balanced salt solution alone. The activity of lipoprotein lipase remained unchanged, since the recovery of total lipoprotein lipase activity added to the incubation medium did not differ from that of untreated cells in either case (98 and 100% of untreated cells). It is therefore concluded that the removal of heparan sulfate from the cell surface interfered with the binding of lipoprotein lipase by endothelial cells, but the removal of chondroitin sulfate had no effect on the lipoprotein lipase binding ability of cells.

Exposure of ^{35}S -labeled cells to heparin. Possible release of [^{35}S]glycosaminoglycans from endothelial cells by incubation with heparin was determined. In this experiment, ^{35}S -prelabeled endothelial cell cultures were incubated with heparin at room temperature for 5 min. As noted in Fig. 3, these conditions result

TABLE II
Treatment of Endothelial Cells with Pure Heparinase and Chondroitin ABC Lyase

Treatment	Surface [^{35}S]glycosaminoglycan remaining (n = 4)	Lipoprotein lipase bound to cells (n = 3)
	%	
Pure heparinase, 2.4 U/ml*	39 \pm 3	15 \pm 3
Chondroitin ABC lyase, 60 U/ml*	35 \pm 3	75 \pm 11
Hanks' balanced salt solution	77 \pm 2	77 \pm 7

Mean \pm SE. n, number of determinations. Data are expressed as the percentage of those for untreated cells. See legend for Fig. 4.

* Unitage is in micromoles per hour of unsaturated double-bond formed rather than micromoles per minute as defined by Saito et al. (22).

in release of lipoprotein lipase from cells. With heparin concentration of $\leq 60 \mu\text{g/ml}$, there was release of no more than 3% of the total [^{35}S]glycosaminoglycans, which did not differ from incubations without heparin. These results indicate that the release of lipoprotein lipase with heparin was not associated with a release of glycosaminoglycans from the cell surface.

DISCUSSION

Our studies show that cultured endothelial cells do not contain lipoprotein lipase, which is consistent with previously reported data (24). Since the enzyme can be synthesized by other isolated cells such as adipocytes (25) and heart cells (26, 27), it has been postulated that cells other than the endothelium are the source of lipoprotein lipase and that the enzyme is transferred to the endothelium, where it binds to the plasma membrane (2, 12, 13). Despite these theories regarding interactions between lipoprotein lipase and the endothelial cell, there has been no previous direct study of the binding of this enzyme to endothelial cells, of the mechanism of the binding, or of the release of lipoprotein lipase from endothelial cells by heparin in systems in vitro.

Lipoprotein lipase from bovine milk is thought to be structurally similar to that appearing in circulating plasma after heparin injection (28, 29). Our data showed that purified lipoprotein lipase bound to cultured endothelial cells and that the bound lipoprotein lipase was released from cells by relatively low concentrations of heparin. The relative displacements of lipoprotein lipase from endothelial cells by various glycosaminoglycans in our experiments follow a pattern similar to that previously noted by Bengtsson et al. (7), who studied both the release of lipoprotein lipase from a Sepharose gel, where it had been bound through heparin or other polysaccharide linkage, and the release of lipoprotein lipase in vivo by intravenous injection of polysaccharide preparations. These data provide an indirect support for the concept of lipoprotein lipase binding to the endothelial cell through glycosaminoglycans at the cell surface and release of lipoprotein lipase by heparin through a detachment from these glycosaminoglycans.

Recent studies have indicated that under certain conditions endogenous heparan sulfate is released, at least in part, from cells such as rat hepatocytes (30) and Chinese hamster ovarian cells (31) on addition of heparin. Among various glycosaminoglycans, only heparin and heparan sulfate, but none of the other polysaccharides, were able to displace the endogenous polysaccharide (30). Under the conditions used in the present study, however, the release of lipoprotein lipase by heparin was not associated with a release of heparan sulfate or other glycosaminoglycans from

the endothelial cells. Therefore, we would suggest that the lipoprotein lipase release by heparin injection in vivo does not involve a release of a glycosaminoglycan-lipoprotein lipase complex but probably represents a detachment of the lipoprotein lipase from the glycosaminoglycan of the cell surface. Our data showed that the enzymatic activity of lipoprotein lipase bound to endothelial cells did not change after release by heparin. This agrees with the findings of Fielding and Higgins (32) that the kinetic properties of the lipoprotein lipase of the perfused rat heart endothelium are the same as for the enzyme after its release by heparin.

The experiments with ^{35}S -labeled endothelial cell cultures showed the removal of up to 90% of sulfated glycosaminoglycans from the cell surface by treatments with crude *F. heparinum* enzyme. The reduction of lipoprotein lipase binding to cells occurred in parallel with the removal of sulfated glycosaminoglycans from the cell surface. The *F. heparinum* enzyme removes glycosaminoglycans from cell surfaces without causing apparent injury or detachment of cells (14). Furthermore, the cells treated with *F. heparinum* enzyme do not show any degradation of cellular protein and are able to continue normal glycosaminoglycan synthesis (14). Hence, it is very unlikely that the interference with binding by this treatment is due to proteinase activity in *F. heparinum* enzyme preparations.

To test the effect of the selective removal of these glycosaminoglycans, we used purified heparinase, which does not degrade chondroitin sulfate (33) and chondroitin ABC lyase, which does not degrade heparan sulfate (22). The use of these enzymes showed that binding of lipoprotein lipase was almost completely inhibited when cells were treated with purified heparinase, but there was no influence on binding of lipoprotein lipase after the treatment of cells with chondroitin ABC lyase. Thus, this approach indicates that heparan sulfate is the glycosaminoglycan through which lipoprotein lipase attaches to the cell surface.

Lipoprotein lipase may serve as a prototype of other plasma components such as platelet factor 4, some clotting factors, antithrombin III, and lipoproteins that also have the capability to bind heparin and other polysaccharides (8). It is possible that heparan sulfate on the endothelial cell surface plays a role in the interactions of the vascular surface with these components, as well as with lipoprotein lipase. Such an interaction has already been described for platelet factor 4 (34).

ACKNOWLEDGMENTS

The authors wish to thank Ms. Wendy Baur for her expert technical assistance related to cell cultures.

This work was supported in part by research grants HL-06924, HL-14456, and AM-08816 from the National In-

stitutes of Health and by the Medical Research Service of the Veterans Administration. Dr. Shimada was a recipient of the Parker B. Francis Foundation Fellowship Award in Pulmonary Research.

REFERENCES

1. Robinson, D. S. 1970. The function of the plasma triglycerides in fatty acid transport. *Compr. Biochem.* 18: 51-116.
2. Scow, R. O., E. J. Blanchette-Mackie, and L. C. Smith. 1976. Role of capillary endothelium in the clearance of chylomicrons. A model for lipid transport from blood by lateral diffusion in cell membranes. *Circ. Res.* 39: 149-162.
3. Korn, E. D. 1955. Clearing factor, a heparin-activated lipoprotein lipase. I. Isolation and characterization of the enzyme from normal rat heart. *J. Biol. Chem.* 215: 1-14.
4. Olivecrona, T., T. Egelrud, P.-H. Iverius, and U. Lindahl. 1971. Evidence for an ionic binding of lipoprotein lipase to heparin. *Biochem. Biophys. Res. Commun.* 43: 524-529.
5. Bengtsson, G., and T. Olivecrona. 1977. Interaction of lipoprotein lipase with heparin-Sepharose. Evaluation of conditions for affinity binding. *Biochem. J.* 167: 109-119.
6. Iverius, P.-H., U. Lindahl, T. Egelrud, and T. Olivecrona. 1972. Effects of heparin on lipoprotein lipase from bovine milk. *J. Biol. Chem.* 247: 6610-6616.
7. Bengtsson, G., T. Olivecrona, M. Höök, J. Riesenfeld, and U. Lindahl. 1980. Interaction of lipoprotein lipase with native and modified heparin-like polysaccharides. *Biochem. J.* 189: 625-633.
8. Lindahl, U., and M. Höök. 1978. Glycosaminoglycans and their binding to biological macromolecules. *Annu. Rev. Biochem.* 47: 385-417.
9. Buonassisi, V. 1973. Sulfated mucopolysaccharide synthesis and secretion in endothelial cell cultures. *Exp. Cell Res.* 76: 363-368.
10. Buonassisi, V., and M. Root. 1975. Enzymatic degradation of heparin-related mucopolysaccharides from the surface of endothelial cell cultures. *Biochim. Biophys. Acta.* 385: 1-10.
11. Wasteson, Å., B. Glimelius, C. Busch, B. Westermarck, C.-H. Heldin, and B. Norling. 1977. Effect of a platelet endoglycosidase on cell surface associated heparan sulphate of human cultured endothelial and glial cells. *Thromb. Res.* 11: 309-321.
12. Olivecrona, T., G. Bengtsson, S.-E. Marklund, U. Lindahl, and M. Höök. 1977. Heparin-lipoprotein lipase interactions. *Fed. Proc.* 36: 60-65.
13. Nilsson-Ehle, P., A. S. Garfinkel, and M. C. Schotz. 1980. Lipolytic enzymes and plasma lipoprotein metabolism. *Annu. Rev. Biochem.* 49: 667-693.
14. Gill, P. J., J. Adler, C. K. Silbert, and J. E. Silbert. 1981. Removal of glycosaminoglycans from cultures of human skin fibroblasts. *Biochem. J.* 194: 299-307.
15. Ryan, U. S., E. Clements, D. Habliston, and J. W. Ryan. 1978. Isolation and culture of pulmonary artery endothelial cells. *Tissue & Cell.* 10: 535-554.
16. Polsky-Cynkin, R., and B. L. Fanburg. 1979. Immunological comparison of angiotensin I converting enzyme from different rat organs. *Int. J. Biochem.* 10: 669-674.
17. Iverius, P.-H. 1971. Coupling of glycosaminoglycans to agarose beads (Sepharose 4B). *Biochem. J.* 124: 677-683.
18. Iverius, P.-H., and A.-M. Östlund-Lindqvist. 1976. Lipoprotein lipase from bovine milk. Isolation procedure, chemical characterization, and molecular weight analysis. *J. Biol. Chem.* 251: 7791-7795.
19. Weber, K., and M. Osborn. 1963. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* 244: 4406-4412.
20. Nilsson-Ehle, P., and M. C. Schotz. 1976. A stable, radioactive substrate emulsion for assay of lipoprotein lipase. *J. Lipid Res.* 17: 536-541.
21. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 193: 265-275.
22. Saito, H., T. Yamagata, and S. Suzuki. 1968. Enzymatic methods for the determination of small quantities of isomeric chondroitin sulfates. *J. Biol. Chem.* 243: 1536-1542.
23. Fielding, C. J. 1968. Inactivation of lipoprotein lipase in buffered saline solutions. *Biochim. Biophys. Acta.* 159: 94-102.
24. Howard, B. V. 1977. Uptake of very low density lipoprotein triglyceride by bovine aortic endothelial cells in cultures. *J. Lipid Res.* 18: 561-571.
25. Björntorp, P., M. Karlsson, H. Pertoft, P. Pettersson, L. Sjöström, and U. Smith. 1978. Isolation and characterization of cells from rat adipose tissue developing into adipocytes. *J. Lipid Res.* 19: 316-324.
26. Henson, L. C., M. C. Schotz, and I. Harary. 1977. Lipoprotein lipase in cultured heart cells: characteristics and cellular location. *Biochim. Biophys. Acta.* 487: 212-221.
27. Chajek, T., O. Stein, and Y. Stein. 1978. Lipoprotein lipase of cultured mesenchymal rat heart cells. I. Synthesis, secretion, and releasability by heparin. *Biochim. Biophys. Acta.* 528: 456-465.
28. Hernell, O., T. Egelrud, and T. Olivecrona. 1975. Serum-stimulated lipases (lipoprotein lipases). Immunological crossreaction between the bovine and the human enzymes. *Biochim. Biophys. Acta.* 381: 233-241.
29. Östlund-Lindqvist, A.-M. 1979. Properties of salt-resistant lipase and lipoprotein lipase purified from human post-heparin plasma. *Biochem. J.* 179: 555-559.
30. Kjellén, L., Å. Oldberg, and M. Höök. 1980. Cell-surface heparan sulfate. Mechanisms of proteoglycan-cell association. *J. Biol. Chem.* 255: 10407-10413.
31. Kraemer, P. M. 1977. Heparin releases heparan sulfate from the cell surface. *Biochem. Biophys. Res. Commun.* 78: 1334-1340.
32. Fielding, C. J., and J. M. Higgins. 1974. Lipoprotein lipase: comparative properties of the membrane-supported and solubilized enzyme species. *Biochemistry.* 13: 4324-4330.
33. Linker, A., and P. Hovingh. 1972. Heparinase and Heparitinase from Flavobacteria. *Methods Enzymol.* 28: 902-911.
34. Busch, C., J. Dawes, D. S. Pepper, and Å. Wasteson. 1980. Binding of platelet factor 4 to cultured human umbilical vein endothelial cells. *Thromb. Res.* 19: 129-137.