Heparin Binds to Human Monocytes and Modulates Their Procoagulant Activities and Secretory Phenotypes. Effect of Histidine-Rich Glycoprotein

By Lawrence Leung, Katsuyasu Saigo, and Deborah Grant

The binding of heparin to human monocytes and the monocytoid cell line U937 was characterized. Heparin binding was rapid, specific, saturable, and reversible. There was a single class of heparin binding sites, with an apparent dissociation constant of 0.19 μmol/L and 1.9 x 10⁶ sites per cell. The binding was not dependent on the anticoagulant property of heparin. Analysis of surface-iodinated cell lysates by heparin affinity chromatography revealed a major 120 Kd cell surface heparin-binding protein. Histidine-rich glycoprotein, a potent heparin antagonist found in human plasma and platelets, decreased the affinity of heparin for cell binding. Cell surface bound heparin was functionally active and markedly accelerated the inactivation of thrombin by antithrombin III. Heparin induced the release of two monocyte secretory proteins of 160 and 17 Kd. Our study supports the thesis that heparin and related glycosaminoglycans interact with monocytes and macrophages, as well as endothelial cells and smooth muscle cells, and play an important and complex role in blood vessel wall biology.

MATERIALS AND METHODS

Carrier free [35]Iodine 131 and 35S-methionine were purchased from New England Nuclear, Boston. Phenylmethylsulfonyl fluoride (PMSF), benzamidine, soybean trypsin inhibitor (SBTI), and phenol 12-myristate 13-acetate (PMA) were purchased from Sigma Chemical Co, St Louis. Paraamphenophenyl paraguanidinobenzoate (pNGB) was purchased from ICN Biomedical Inc (Costa Mesa, CA). Phenylalanyl-prolyl-arginyl chloromethyl ketone (PPACK) and 3-[(3-cholamidopropyl)-dimethyl ammonio]-1-propane sulfonate (CHAPS) were purchased from Calbiochem-Behring Corp, La Jolla, CA. Thrombin substrate S2238 was purchased from Helena Laboratory (Beaumont, TX). Heparin-Sepharose CL 4B and a fast-protein liquid chromatography (FPLC) system, including an anion-exchange Mono-Q column, were purchased from Pharmacia, Piscataway, NJ. Microtitration plates and a Titertek multiscan photometer were purchased from Flow Laboratories, Inc, McLean, VA. All reagents were of analytical grade.

From the Department of Medicine, Division of Hematology, Stanford University School of Medicine, CA.

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Dr Leung is an Established Investigator of the American Heart Association.

Address reprint requests to Lawrence Leung, MD, Division of Hematology, S-161, Stanford University Medical School, Stanford, CA 94305.

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52 (60 g/L plasma) at room temperature for 30 minutes. The CM-cellulose was washed extensively with distilled water, then batch eluted with 0.5 mol/L NH$_4$HCO$_3$. The eluate was dialyzed overnight against 0.02 mol/L phosphate-buffer containing 0.4 mol/L NaCl, pH 6.3, then applied to a heparin-sepharose 6B column preequilibrated with the same buffer. After extensive washings, the column was eluted with phosphate buffer containing 1.0 mol/L NaCl. HRPG eluted from the heparin column was >92% pure as judged by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and laser densitometry, with mol wt 63,000 reduced and 74,000, reduced, as previously reported.24'3 Microsequencing of the purified protein revealed that the N-terminal 15 residues were identical to that published.28

Quenching of the purified protein revealed that the N-terminal IS residues were identical to that published.28

Trypsin treatment at this concentration was not effective and did not cause any RBC lysis and did not interfere with the absorbance measurement. The reaction mixtures (total volume 200 µL) were centrifuged and the absorbance of the supernates read at 405 nm. Each experiment was repeated three times with each data point done in duplicate.

**Heparin binding to U937 cells.**

Monocytes and U937 cells (7 x 10$^6$ cells/mL) were incubated in 20 mmol/L phosphate buffered saline, pH 7.4, containing 2% bovine serum albumin (PBS/BSA) for one hour at 4°C. $^{35}$S-heparin, in the presence or absence of 50-fold excess of unlabeled heparin, was added to cell aliquots and incubated for one hour at 4°C. The final volume of the reaction mixture was 300 µL. With a cell concentration of 3.6 x 10$^6$ cells/mL. At the end of incubation, cell-bound heparin was separated from unbound heparin by centrifuging the cell suspensions through silicone oil (6.7 parts Dow Corning oil 550, 3.3 parts Dow Corning oil 556, S.G. 1.040) at 14,000 g for ten minutes in a Beckman microfuge. The cell pellets were solubilized with 0.2 N NaOH, added to Biofluor, and cell-bound radioactivity determined in a scintillation counter. Each experiment was repeated at least three times with each data point performed in duplicate.

**Internalization of bound heparin by U937 cells.**

Aliquots of U937 cells were incubated separately in PBS/BSA buffer for one hour at 4°C and 37°C. Binding of $^{35}$S-heparin (final concentration, 5 µmol/L) was carried out at the two temperatures for three hours. At the end of incubation, cell suspensions were washed with cold PBS/BSA and treated with trypsin (0.25%) for 30 minutes at 4°C. Cell-bound heparin, in the presence or absence of trypsin treatment, was determined. Trypsin treatment at this concentration did not cause any cell death as determined by trypan blue exclusion. Alternatively, instead of surface trypsinization, internalized heparin was determined by using 50-fold excess of unlabeled heparin to displace surface-bound $^{35}$S-heparin.

**Fractionation of $^{35}$S-heparin by ATIII affinity chromatography.**

Nine milligrams of purified ATIII was coupled to 2.3 mL of CNBr-activated sepharose 4B. Aliquots of $^{35}$S-heparin were applied to the ATIII-sepharose column in PBS and eluted with 20 mmol/L phosphate buffer, pH 7.4, containing 2 mol/L NaCl.4$^{1}$The column was eluted with fractions (low-affinity heparin) and the eluted fractions (high-affinity heparin) were separated pooled, dialyzed, and concentrated.

**Heparin affinity chromatography of surface iodinated U937 cell lysates.**

U937 cells were surface iodinated using the immobilized lactoperoxidase bead method (BioRad, Richmond, CA). The labeled cells were washed with phosphate buffer three times and solubilized by 15 mmol/L CHAPS with protease inhibitors at 4°C for one hour. After centrifugation to remove any insoluble materials, aliquots of the cell lysates were applied to a heparin-sepharose 6B column and, after extensive washings, eluted with 1 mol/L NaCl. The eluates were analyzed by SDS-PAGE and autoradiography.

**Inactivation of thrombin by heparin-pretreated U937 cells.**

U937 cells and control RBC were first incubated with RPMI/BSA at 4°C for one hour, then incubated with or without heparin (final concentration, 0.24 µmol/L) at 4°C for one hour. Cells were washed once with PBS/BSA and fixed with glutaraldehyde (final concentration, 0.1%) for 20 minutes at room temperature. After fixation, the cells were washed with PBS three times. Using heparin-sepharose beads, it was shown that glutaraldehyde fixation at this concentration did not affect the heparin enhancement effect on thrombin inactivation by ATIII. The fixed U937 cells and RBC, with or without heparin preincubation, were added in various concentrations to thrombin (final concentration, 10 U/mL) and ATIII (final concentration, 10 µg/mL) in 40 mmol/L Tris-HCl, 0.15 mol/L NaCl, pH 8.3. After 30 seconds of incubation, thrombin substrate S2238 (final concentration, 0.5 mmol/L) was added. After another 30 seconds of incubation, acetic acid (final concentration, 15.6%) was added to stop the reaction. Acetic acid did not cause any RBC lysis and did not interfere with the absorbance measurement. The reaction mixtures (total volume 200 µL) were centrifuged and the absorbance of the supernates read at 405 nm. Each experiment was repeated three times with each data point done in duplicate.

**Heparin-HRGP interaction.**

HRGP (final concentration, 10 µg/mL) was coated on microtitration wells as previously described.28 Unbound HRGP was removed with washing with Tris/Tween buffer (10 mmol/L Tris-HCl, 0.15 mol/L NaCl, 0.05% Tween 20, pH 7.4) three times. $^{125}$I-heparin in Tris/Tween buffer containing 2 mmol/L CaCl$_2$ or 2 mmol/mL EDTA, in the presence or absence of 50-fold excess of unlabeled heparin, was added and incubated for one hour at 37°C. After extensive washings with Tris/Tween buffer, the HRGP-bound heparin was solubilized with 0.2 N NaOH, added to Biofluor, and radioactivity determined. Alternatively, heparin-sepharose beads were incubated with Tris-saline/2% BSA, followed by $^{125}$I-HRGP in the same buffer containing 2 mmol/L CaCl$_2$ or 2 mmol/L EDTA, for one hour at room temperature. The samples were placed into modified 1-mL Eppendorf pipette tips fitted with filter plugs (U-11 plug; Forex Technologies, Fairburn, GA). Unbound proteins were separated by centrifugation for one minute at 2,500 rpm in a table-top centrifuge. Heparin beads retained on the filters were washed three times with Tris/Tween buffer and the amount of $^{125}$I-HRGP bound counted.

**Metabolic labeling of monocytes.**

Isolated human peripheral blood monocytes were incubated in tissue culture wells at 1 to 2 x 10$^6$ cells/mL. After two hours of incubation, the nonadherent cells were removed and the adherent cells cultured in heparin for various periods of time. For the last two hours of heparin incubation, the culture media was replaced by methionine-free RPMI with $^{35}$S-methionine (40 µCi/mL) and 0.4% FCS. At the end of incubation, the culture media were removed and precipitated with 10% trichloroacetic acid. The precipitates were dissolved in sample buffer and analyzed by SDS-PAGE and autoradiography.

**RESULTS**

**Heparin binding to U937 cells and human monocytes.**

$^{38}$S-heparin bound to U937 cells in a concentration-dependent and saturable manner. Nonspecific binding, as determined by binding in the excess of unlabeled heparin, was <10% (Fig 1). Similar specific binding of heparin to isolated human peripheral blood monocytes was observed...
Dependent. The binding reached a steady state at 30 minutes. The binding of heparin to U937 cells was not inhibited by 2 mmol/L EDTA, indicating that it was not divalent cation dependent. The specific heparin binding to U937 cells was analyzed. The concentration of RBCs was fivefold greater than that of U937 cells (data not shown). Heparin binding to control human RBCs was minimal, demonstrating the relative specificity of binding for U937 cells and human monocytes. In these studies, the concentration of RBCs was fivefold greater than that of U937 cells in order to provide an equivalent cell surface area. The binding of heparin to U937 cells was not inhibited by 2 mmol/L EDTA, indicating that it was not divalent cation dependent. The binding reached a steady state at 30 minutes at 4°C and was reversible, with >90% of the surface bound labeled heparin displaced by excess unlabeled heparin (Fig 2). Scatchard plot analysis of the specific heparin binding revealed a single class of heparin binding sites, with an apparent dissociation constant (kd) of 0.19 μmol/L, and 1.92 x 10^6 binding sites per cell (Fig 1, insert).

To determine the specificity of binding for heparin, binding of 35S-heparin was performed in the presence of excess unlabeled heparin, dermatan sulfate, and chondroitin-4-sulfate at 4°C. Cell-bound radioactivity was then determined. 35S-heparin bound in the absence of unlabeled glycosaminoglycans was considered 100% binding.

Fig 2. Time course and reversibility of heparin binding to U937 cells. U937 cells were incubated in PBS/BSA for one hour at 4°C. 35S-heparin (final concentration, 0.5 μmol/L) was added. Duplicate aliquots of the cell suspensions were removed at various time points and spun through silicone oil to determine cell-bound radioactivity (A). After 30 minutes incubation with 35S-heparin, 100-fold excess unlabeled heparin was added and the cell-bound radioactivity at different time intervals determined (B).

Fig 3. Specificity of heparin binding to U937 cells. U937 cells were incubated with PBS/BSA for one hour at 4°C. 35S-heparin (final concentration, 0.5 μmol/L) was added in the presence of various molar excess of unlabeled heparin, dermatan sulfate, and chondroitin-4-sulfate for 30 minutes at 4°C. Cell-bound radioactivity was then determined. 35S-heparin bound in the absence of unlabeled glycosaminoglycans was considered 100% binding.

Table 1. Internalization of Surface-Bound Heparin by U937 Cells

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Trypsin</th>
<th>Heparin Bound (ng/10^6 cells)</th>
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<tbody>
<tr>
<td></td>
<td>4°C</td>
<td>37°C</td>
</tr>
<tr>
<td>1 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−</td>
<td>56.4 ± 2.8</td>
<td>45.2 ± 3.3</td>
</tr>
<tr>
<td>+</td>
<td>5.1 ± 0.3*</td>
<td>8.5 ± 0.6*</td>
</tr>
<tr>
<td>3 h</td>
<td></td>
<td></td>
</tr>
<tr>
<td>−</td>
<td>51.9 ± 3.1</td>
<td>39.4 ± 2.7</td>
</tr>
<tr>
<td>+</td>
<td>5.8 ± 0.4†</td>
<td>11.0 ± 1.4†</td>
</tr>
</tbody>
</table>

U937 cells were incubated in PBS/BSA buffer for one hour at 4°C and 37°C. Binding of 35S-heparin (final concentration 5 μmol/L) was carried out at the two temperatures for one hour and three hours. At the end of incubation, cell suspensions were washed with cold PBS/BSA and trypsinized (0.25%) at 4°C for 30 minutes. Cell-bound heparin was then determined. Data presented were the mean ± SD (n = 8).

*P ≤ .01. †P ≤ .01.
Internalization of bound heparin by U937 cells. To determine if the bound heparin was internalized by U937 cells, binding of $^{35}$S-heparin was carried out at 4°C and 37°C. Cells were then trypsinized and the residual amount of cell-bound heparin determined (Table 1). At 4°C, ~10% of the cell-bound heparin was not removed by surface trypsinization, consistent with the ~10% nonspecific binding as determined by excess unlabeled heparin, indicating that there was minimal cellular internalization of heparin at 4°C. When binding was performed at 37°C, ~18% and 28% of the respective total cell-bound heparin at one hour and three hours of incubation was not removed by trypsin, demonstrating internalization of heparin by U937 cells at 37°C. Alternatively, when excess unlabeled heparin was added to displace $^{35}$S-heparin from the cell surface, a similar increase in the amount of nondisplaceable cell-bound heparin was observed at 37°C compared with 4°C (data not shown).

Binding of heparin fractions to U937 cells. To further characterize the heparin binding to U937 cells, $^{35}$S-heparin was separated into fractions with high or low affinity for ATIII by ATIII affinity chromatography. While the binding of the high-affinity heparin fraction was equivalent to the unfractionated heparin, there was also significant binding of the low-affinity heparin fraction to U937 cells, indicating that the binding was not dependent on the anticoagulant property of heparin (Table 2). Binding of the unfractionated $^{35}$S-heparin was also performed in the presence of 20-fold excess of unlabeled heparin preparations with various mol wts and anticoagulant activities. A low mol wt heparin (RD heparin, mol wt 5,127, 98 USP U/mL) was as effective as regular heparin in inhibiting $^{35}$S-heparin binding (94% inhibition). A heparin fragment with minimal anticoagulant activity (DH heparin, mol wt 2,750, 7 USP U/mL) also caused significant inhibition (73% inhibition), consistent with the observation that heparin binding was not dependent on its anticoagulant property.

Characterization of heparin-binding proteins on U937 cell surface. Detergent-solubilized cell surface iodinated U937 cell lysates were applied to a heparin-sepharose affinity column and analysis of the eluate revealed a major cell surface heparin-binding protein with mol wt 120,000 (Fig 4). When the cell lysates were applied in the presence of excess fluid phase heparin, the 120 Kd protein was not detected. Additional control using an albumin-sepharose column was also negative, demonstrating the specificity of this heparin-binding protein. The identity of the 120 Kd cell surface protein was not determined.

Modulation of heparin binding by HRGP. HRGP is a

<table>
<thead>
<tr>
<th>Table 2. Binding of Heparin Fractions to U937 Cells</th>
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<tr>
<td>Heparin Bound (ng/10^6 cells)</td>
</tr>
<tr>
<td>Unfractionated heparin 42.7</td>
</tr>
<tr>
<td>High affinity heparin 40.5</td>
</tr>
<tr>
<td>Low affinity heparin 26.8</td>
</tr>
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</table>

Labeled heparin was separated into high and low affinity fractions by ATIII affinity chromatography. Heparin binding (final concentration, 0.4 μmol/L) was performed as described in Fig 1. Data presented are the mean of two different experiments.

Fig 4. Heparin-binding proteins on U937 cells. Detergent lysates of surface-iodinated U937 cells were applied in the presence or absence of excess heparin, to a heparin-sepharose column, and after extensive washings, eluted with 1 mol/L NaCl. The eluates were analyzed by SDS-PAGE and autoradiography. Lane 1, surface-iodinated U937 cell lysates; lane 2, heparin column eluate with lysates applied in the presence of excess heparin; lane 3, heparin column eluate with lysates applied in the absence of fluid phase heparin; lane 4, eluate from control albumin column.

Fig 5. Effect of HRGP on heparin binding to U937 cells. The binding of $^{35}$S-heparin to U937 cells in the presence of different concentrations of HRGP was performed. A, no HRGP; B, 50 μg/ml HRGP; C, 100 μg/ml HRGP.
potent heparin-binding protein found in plasma and is released from activated platelets. Whether HRGP would modulate heparin binding to U937 cells was examined. HRGP caused a concentration-dependent shift of the heparin binding isotherm to the right, requiring a fourfold increase in heparin concentration to achieve 50% binding in the presence of 100 µg/mL HRGP. The data suggest that HRGP significantly decreased the affinity of heparin for U937 cells (Fig 5). The inhibitory effect was not due to contamination of the HRGP samples by heparin during the preparative procedure since HRGP that had been further purified by anion-exchange (Mono-Q) chromatography had a similar effect on heparin cell binding. While the heparin interaction with HRGP was dependent on divalent cation and inhibited by EDTA (Fig 6A) as previously reported, EDTA did not abolish the HRGP inhibitory effect on heparin binding to U937 cells (Fig 6B).

**Heparin binding to PMA-stimulated U937 cells and modulation by HRGP.** Heparin bound to PMA-stimulated U937 cells with similar characteristics as nonstimulated cells, with a slight decrease in the affinity of heparin binding (50% binding occurring at 0.9 µmol/L instead of 0.4 µmol/L), and a 20% decrease in total heparin cell binding. HRGP also partially inhibited heparin binding to PMA-stimulated cells (data not shown).

**Inactivation of thrombin by heparin-pretreated U937 cells.** To determine the functional role of cell-bound heparin, the effect of heparin-pretreated U937 cells on thrombin inactivation by ATIII was investigated (Table 3). U937 cells not pretreated with heparin had no effect on ATIII inactivation of thrombin. However, pretreatment with heparin markedly accelerated the inactivation of thrombin by ATIII, indicating that the cell-bound heparin was anticoagulantly functional. Using labeled heparin, it was demonstrated that the enhancement effect was not due to the release of cell-bound heparin into the fluid phase. Control studies with human RBC pretreated with heparin did not show any enhancement effect.

<table>
<thead>
<tr>
<th>Reaction Mixture</th>
<th>A450</th>
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<tbody>
<tr>
<td>Thrombin</td>
<td>0.494</td>
</tr>
<tr>
<td>Thrombin + ATIII</td>
<td>0.344</td>
</tr>
<tr>
<td>Thrombin + ATIII + U937</td>
<td>0.368</td>
</tr>
<tr>
<td>Thrombin + ATIII + Heparin/U937</td>
<td>0.085</td>
</tr>
<tr>
<td>Thrombin + ATIII + Heparin/RBC</td>
<td>0.390</td>
</tr>
</tbody>
</table>

In the presence of 100 µg/mL HRGP, the enhancement effect was not abolished.

**Effect of heparin on monocyte secretory proteins.** When metabolically labeled human monocytes were incubated with heparin, an increase of two secretory proteins, mol wt 160,000 and 17,000, in the culture media was observed (Fig 8). The inductive effect of heparin was dose dependent and occurred quite rapidly (after two hours) with maximal induction observed after 12 to 14 hours. A low mol wt heparin with minimal anticoagulant activity (DH heparin, 7 USP U/mL) was also effective in the induction of these two secretory proteins, whereas dermatan sulfate was much less effective. Of note, the heparin inductive effect was not...
concentrations of observed secretory proteins remained to be determined. and residual thrombin activities determined. mixture was cells/mi U937 cells. Heparin-pretreated U937 cells at pretreated in Table 2. (B) Inactivation of thrombin by heparin-described ATIII (10 g/mL) and residual thrombin activities determined as applied in each lane. and reversible, suggesting a ligand-receptor type interaction was specific, time- and concentration-dependent, saturable, and reversible, suggesting a ligand-receptor type interaction of the cell surface (Figs 1 through 3). Since heparin is highly negatively charged, a strong ionic interaction between heparin and cell surface proteins with basic characteristics is anticipated, raising the possibility that heparin may bind to multiple cell surface proteins. However, Scatchard plot analysis of the binding data revealed a single class of heparin binding sites (Fig 1), and heparin affinity chromatography of the surface labeled cell lysates revealed a major 120 Kd heparin-binding protein (Fig 4). These findings demonstrate that heparin only bound to a limited number of specific cell surface proteins. Whether the 120 Kd heparin-binding protein represents a heparin receptor on the monocyte cell surface remains to be determined.

The number of heparin-binding sites on U937 cells (1.9 × 10⁶ binding sites per cell) is in the same range as that reported for human endothelial cells (1 × 10⁶ sites per cell) and rat liver cells (1 × 10⁵ sites per cell),۵۳۴ and higher than that found on smooth muscle cells (1 × 10⁵ sites per cell).۵۷ Heparin binding to mouse peritoneal macrophages has also been described but the affinity of heparin binding and the number of binding sites were not reported.۵۸ More recently, heparin binding to human platelets is observed.۵۹۶۰ In view of the present findings, it will be of interest to characterize the cell-surface heparin-binding proteins from these various cell types and determine if they are structurally and functionally related.

HRGP, a potent heparin antagonist found in human plasma and released from activated platelets,۵۱۶۱ decreased the affinity of heparin for U937 cells (Fig 5). Significant modulation of heparin binding was observed with HRGP concentration at 50 to 100 μg/mL, suggesting that plasma HRGP, at 100 to 150 μg/mL,۵۲۵۳ would have a similar effect. Recently, HRGP inhibition of the antiproliferative effect of heparin on arterial smooth muscle cells was reported.۵۱ Taken together, the data suggest that the modulation of heparin binding to cells by HRGP may represent a major function for this protein in vivo.

The mechanism whereby HRGP modulates heparin binding to the monocyte cell surface is not fully defined. The observation that HRGP decreased the affinity of heparin binding suggests that the HRGP-binding site is different from the cell-binding site on the heparin molecule. It should be noted that while EDTA abolished the direct HRGP-heparin interaction as previously reported,۵۴ it had no effect on the HRGP inhibition of heparin cell binding (Fig 6). The data suggest that, in addition to competition for heparin binding in the fluid phase, HRGP may interact directly with the cell surface and thereby alter its affinity for heparin. Recently, we obtained data demonstrating direct binding of HRGP to U937 cells (K. Saigo, L. Leung, unpublished observation, July 1987).

Cell surface bound heparin was functionally active, and markedly accelerated the inactivation of thrombin by ATIII (Table 3). The anticoagulant potency of the cell-bound heparin was equivalent to that of fluid phase heparin (Fig 7). Human monocytes and macrophages generate potent tissue-factor activity on stimulation and provide an optimal cell surface for the efficient assembly of prothrombinase, the major enzyme complex that catalyzes thrombin generation.

**DISCUSSION**

In this study, binding of heparin to human monocytes and the monocytoid cell line U937 was characterized. The binding was specific, time- and concentration-dependent, saturable, and reversible, suggesting a ligand-receptor type interaction observed in U937 cells. The identity of these monocyte secretory proteins remained to be determined.

Adherent human peripheral blood monocytes were cultured in heparin for different incubation times. For the last two hours of incubation, the cells were metabolically labeled with ⁹⁵S-methionine. At the end of incubation, the culture media were removed and precipitated with 10% trichloroacetic acid. The precipitates were dissolved in sample buffer and analyzed by SDS-PAGE and autoradiography. Equal amounts of radioactive counts were applied in each lane.

**Fig 8.** Induction of monocyte secretory proteins by heparin. Adherent human peripheral blood monocytes were cultured in heparin for different incubation times. For the last two hours of incubation, the cells were metabolically labeled with ⁹⁵S-methionine. At the end of incubation, the culture media were removed and precipitated with 10% trichloroacetic acid. The precipitates were dissolved in sample buffer and analyzed by SDS-PAGE and autoradiography. Equal amounts of radioactive counts were applied in each lane.
HEPARIN INTERACTIONS WITH HUMAN MONOCYTES

in vivo.\textsuperscript{42-44} Fibrin deposition is a prominent feature of inflammation and fibrin has been identified on macrophage cell surface.\textsuperscript{42-44} The specific binding and localization of functionally active heparin to the same cells may represent a pathway to modulate the procoagulant properties of monocytes and macrophages at sites of inflammation and thrombosis.

The functional consequence of heparin binding to monocytes is not limited to its anticoagulant property. Significant binding of heparin fractions with low affinity for ATIII to U937 cells was demonstrated (Table 2). Heparin induced the release of two monocyte secretory proteins of 160 and 17 Kd (Fig 8). A similar heparin augmentation effect on the secretion of smooth muscle cell proteins has been observed.\textsuperscript{12,44} Heparin stimulates macrophages, including the release of a mononuclear cell factor, interferon,\textsuperscript{49} and the promotion of lysosomal enzyme secretion.\textsuperscript{50} It is of note that native heparin from mast cells also has low anticoagulant activity\textsuperscript{55} and that mast cell-macrophage interactions have been implicated in various inflammatory processes in vivo.\textsuperscript{15,25-35} Our study supports the thesis that native heparin and heparin-like glycosaminoglycans, derived from cellular matrices or released from mast cells, interact with monocytes and macrophages as well as endothelial cells and smooth muscle cells, and play an important and complex role in blood vessel wall biology.

ACKNOWLEDGMENT

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Heparin binds to human monocytes and modulates their procoagulant activities and secretory phenotypes. Effects of histidine-rich glycoprotein

L Leung, K Saigo and D Grant