A high molecular weight glycoprotein, reported to be secreted by endothelial cells (Sage, H., Crouch, E., and Bornstein, P. (1979) Biochemistry 18, 5433-5442), has been purified to apparent homogeneity from culture medium of adult bovine aortic endothelial cells. Purification was achieved by ammonium sulfate fractionation and successive chromatography on gelatin-Sepharose, Sephacryl S-300, and hydroxylapatite. The glycoprotein was found to be a disulfide-linked oligomer with a subunit molecular weight of 190,000, as judged by its mobility on sodium dodecyl sulfate (NaDodSO\(_4\)) polyacrylamide gels. The endothelial cell-derived protein is distinct from high molecular weight serum glycoproteins such as fibronectin and α2-macroglobulin. However, immunological and structural studies indicate that the \( M_r = 190,000 \) glycoprotein is either identical with or closely related to thrombospondin, a glycoprotein contained in platelet granules and released in response to thrombin-induced aggregation.

Vascular endothelial cells in culture have been reported to synthesize and secrete fibronectin (1-3) as well as several types of collagen (4-7). Fibronectin and these various collagens contribute to the structural integrity of the extracellular matrix and, thus, may be involved in endothelial cell attachment, maintenance of cell shape, and imposition of polarity in vitro.

Bovine aortic endothelial cells in vitro have also been reported to synthesize and secrete a high molecular weight, disulfide-linked multimeric glycoprotein (6). This glycoprotein was shown to be distinct from fibronectin, although its identity and function were not determined. Since we are interested in characterizing those factors which mediate endothelial cell attachment, migration, and thromboresistance, we have purified this glycoprotein to determine whether it plays a role in any of these cellular processes. The following report describes the structural characterization of this glycoprotein and its apparent identity with thrombospondin, a glycoprotein contained in the α-granule fraction of platelets and released in response to thrombin-induced aggregation (8-11).

**MATERIALS AND METHODS**

**Cell Culture and Metabolic Labeling**—Endothelial cells were isolated from bovine thoracic aorta and cultured as previously described by Schwartz (12). The proteins synthesized by these cells were metabolically labeled with either L-[2-\( ^3 \)H]proline or D-[2-\( ^3 \)H]mannose according to the methods described by Sage et al. (6). Briefly, confluent cultures were exposed to radiolabeled proline or mannose (50 μCi/ml of medium or 80 μCi/10\(^6\) cells) for 24 h in serum-free DMEM supplemented with sodium ascorbate and β-APN. Radiolabeled culture medium was added to a protease inhibitor mixture to produce a final concentration of 0.2 mM PhCH\(_2\)SO\(_3\)O\(_3\)F, 10 mM MalNEt, and 2.5 mM EDTA.

**Fractionation of the Glycoprotein**—The glycoprotein was purified from metabolically labeled endothelial cell culture medium utilizing a combination of ammonium sulfate fractionation and chromatography on gelatin-Sepharose, Sephacryl S-300, and hydroxylapatite. Initially, the radiolabeled culture medium, pooled from 24 tissue culture dishes (150 × 25 mm) of confluent BAE cells, was made 20% (w/v) in ammonium sulfate, and the solution was stirred overnight at 4°C. The precipitated protein was removed by centrifugation at 4000 rpm for 30 min. Ammonium sulfate was removed, and the supernatant was adjusted to a final concentration of 50% (w/v), the solution was stirred overnight at 4°C, and the precipitated protein was removed as described above. The supernatant was discarded, and the pellet was dissolved in 10-15 ml of PBS containing 2.5 mM EDTA and 0.2 mM PhCH\(_2\)SO\(_3\)O\(_3\)F (Buffer A). The sample was then dialyzed against 1 liter of this buffer with a total of three changes over an 18-h period. The retentate was loaded onto a gelatin-Sepharose column (1.5 × 25 cm) (prepared according to the method of Engvall and Ruoslahti (13)), equilibrated in Buffer A, and incubated at least 4 h prior to elution with Buffer A. The flow-through material from the gelatin-Sepharose column was dialyzed against 1 liter of 50 mM ammonium bicarbonate containing 0.2 mM NaDodSO\(_4\) and 0.2 mM PhCH\(_2\)SO\(_3\)F, and then was lyophilized. The lyophilized powder was dissolved in 5-10 ml of PBS containing 3 mM urea, 2.5 mM EDTA, and 0.2 mM PhCH\(_2\)SO\(_3\)O\(_3\)F (Buffer B). Subsequently, the sample was applied to a Sephacryl S-300 column (2 × 100 cm) which had been equilibrated in Buffer B. The sample was eluted from the S-300 column and fractions containing the glycoprotein (identified by slab gel electrophoresis) were pooled, dialyzed against 1 liter of 10 mM NaPO\(_4\) (pH 6.5) containing 3.2 mM urea, 2.5 mM EDTA, and 0.2 mM PhCH\(_2\)SO\(_3\)O\(_3\)F (Buffer C) for 24 h with three buffer changes. The retentate was applied to a hydroxylapatite (Bio-Rad) column (0.9 × 12 cm) which had been equilibrated in Buffer C. The column was washed with 50 ml of equilibration buffer and developed with a linear gradient (between 10 mM and 0.3 M) of sodium phosphate (pH 6.5) which contained 3.2 mM urea, 2.5 mM EDTA, and 0.2 mM PhCH\(_2\)SO\(_3\)O\(_3\)F. Fractions containing the glycoprotein were pooled and concentrated on an Amicon pressure cell using an XM-100A filter.

**NaDodSO\(_4\)-Polyacrylamide Gel Electrophoresis and Fluorescence Autoradiography**—NaDodSO\(_4\) slab gel electrophoresis was performed according to the method of Laemmli (14) as modified by Studier (15), utilizing a 4% stacking gel and a 5% or 6% separating gel. Following electrophoresis, the gels were either stained with 0.25% Coomassie blue or processed for fluorescence autoradiography (16) followed by exposure to sensitized x-ray film (17).

**Notes**

1. The abbreviations used are: DMEM, medium; Dulbecco's modification of Eagle's medium; β-APN, β-Aminopropionitrile; MalNEt, N-ethylmaleimide; BAE, bovine aortic endothelial; PBS, phosphate-buffered saline; NaDodSO\(_4\), sodium dodecyl sulfate; BSA, bovine serum albumin.
observed on the film were quantitated using a scanning densitometer (Beckman DU-8).

**Amino Acid Analysis**—Glycoprotein samples (60-100 μg), to be used for amino acid analysis, were desalted on a Bio-Rad P-2 column (1 X 25 cm) in the presence of 50 mM NH₄HCO₃ (pH 7.8) and then were lyophilized. Amino acid analyses were performed on a Durrum (model Z-2000) amino acid analyzer following 24-h hydrolysis in vacuo in 5.7 N HCl at 110 °C. Cysteine was determined as cysteic acid following performic acid oxidation (18).

**Purification of Thrombospondin**—Human thrombospondin was purified from 2 units of platelet concentrate (obtained from the Puget Sound Blood Center) essentially according to the method of Lawler et al. (10). Minor modifications included the addition of 2.5 mM EDTA to the final resuspension buffer prior to thrombin activation and the termination of the thrombin reaction with 5 mM PhCH₂SO₂F rather than hirudin. Phillips et al. (11) have recently shown that addition of EDTA to the final resuspension buffer significantly enhances the yield of thrombospondin.

Bovine thrombospondin was purified in a similar manner although with more extensive modifications. Bovine blood was collected at the slaughterhouse and rapidly mixed with 0.1 volume of anticoagulant citrate dextrose (3.8 g of trisodium citrate and 2 g of glucose/100 ml of H₂O). The sample was then centrifuged at 300 x g for 6 min at room temperature, followed by several subsequent centrifugations. The platelet-rich plasma was withdrawn and recentrifuged at 300 x g for 6 min in order to remove contaminating red cells. Platelets were isolated by centrifugation at 2,200 x g for 9 min. Platelets were resuspended in 10% anticoagulant dextrose, 20 mM Tris (pH 7.5), 145 mM NaCl, 5 mM KCl, 5 mM glucose, and 25 mM EDTA (0.1 ml/platelet). Highly purified human α-thrombin (a gift from William Canfield, University of Washington, Seattle, WA) was added to a final concentration of 7 units/ml and incubated at room temperature for 15 min in order to initiate platelet aggregation. The reaction was terminated by the addition of PhCH₂SO₂F to a final concentration of 5 mM. Platelet aggregates were removed by centrifugation at 22,000 x g for 9 min to remove residual red cells. Platelets were again pelleted by centrifugation at 2,200 x g for 9 min and subsequently washed twice in Buffer D. Following the final centrifugation, the platelets were resuspended in 20 mM Tris- HCl, pH 7.5, 145 mM NaCl, 5 mM KCl, 5 mM glucose, and 25 mM EDTA (0.1 ml/platelet). Highly purified human α-thrombin (a gift from William Canfield, University of Washington, Seattle, WA) was added to a final concentration of 7 units/ml and incubated at room temperature for 15 min in order to initiate platelet aggregation. The reaction was terminated by the addition of PhCH₂SO₂F to a final concentration of 5 mM. Platelet aggregates were removed by centrifugation at 22,000 x g for 20 min at 4 °C. Eight ml urea was added to the supernatant to a final concentration of 3 M and the sample was applied to a Sephacryl S-300 column as previously described for the purification of the glycoprotein. Protein fractions eluting in the void volume of this column were pooled and chromatographed on hydroxylapatite as previously described. Fractions eluting at a conductivity between 3.5 and 6.5 mmho were pooled and concentrated with an Amicon pressure cell using an XM-100A filter.

**Protein Transfers**—Antibodies to the glycoprotein, purified from bovine aortic endothelial cell media, were raised in rabbits and the serum fractions were enriched by ammonium sulfate fractionation and modified by Sage et al. (23). The two principal contaminating serum proteins were a2-macroglobulin and serum albumin (Fig. 1C). The M₄₊ = 190,000 glycoprotein from serum-free culture media necessitated its separation not only from other proteins synthesized and secreted by the endothelial cells, but also from relatively large amounts of serum proteins that were bound to the endothelial cell layer during subculture and slowly released into the culture medium during the labeling period (Fig. 1C).

The Mᵣ = 190,000 glycoprotein was purified to apparent homogeneity using ammonium sulfate fractionation and chro-
An Endothelial Cell Glycoprotein Related to Thrombospondin

**Fig. 2.** Molecular weight determination of the $M_r = 190,000$ glycoprotein as estimated by electrophoresis in a 6% NaDodSO$_4$-polyacrylamide gel. Molecular weight standards included: unreduced fibronectin (440,000), reduced thyroglobulin (330,000), reduced IgG heavy chains (150,000), reduced phosphorylase (97,000), reduced bovine serum albumin (67,000), and reduced catalase (60,000). Electrophoresis was performed as described under "Materials and Methods." The open circles identify the $M_r = 190,000$ glycoprotein and a $M_r = 160,000$ glycoprotein derived from platelets.

matography on gelatin-Sepharose, Sephacryl S-300, and hydroxylapatite. The 20–50% (w/v) ammonium sulfate precipitation step served to concentrate the $M_r = 190,000$ glycoprotein from the endothelial cell media as well as to separate it from the majority of the type III procollagen. Following the ammonium sulfate fractionation, the sample was applied to a gelatin-Sepharose affinity column (Fig. 3A). The $M_r = 190,000$ glycoprotein did not bind to this column, and greater than 70% of the fibronectin which contaminated the preparation was removed at this point. Subsequent elution of the affinity column with 6 M urea indicated that only fibronectin had bound to the column. The unbound fraction of the gelatin-Sepharose column was pooled and concentrated by lyophilization. The lyophilized protein was redissolved in Buffer B and applied to a Sephacryl S-300 molecular sieve column (Fig. 3B). The $M_r = 190,000$ glycoprotein eluted near the void volume of the column and was clearly separated from serum albumin and from other lower molecular weight contaminants. Chromatography of the pooled Sephacryl S-300 fractions on hydroxylapatite (Fig. 3C) resulted in separation of the glycoprotein from $\alpha_2$-macroglobulin, residual fibronectin, and other high molecular weight contaminants.

Aliquots of the $M_r = 190,000$ glycoprotein preparation, at the various stages of purification, were analyzed by electrophoresis on 6% NaDodSO$_4$-polyacrylamide gels. Figs. 4 and 5 illustrate the Coomassie blue-stained pattern as well as the fluorescence autoradiogram of such an electrophoretic analysis. These results are representative of the data gathered from 26 separate preparations of the $M_r = 190,000$ glycoprotein. The preparations appeared to be homogeneous based on

**Fig. 3.** Chromatographic purification of the $M_r = 190,000$ glycoprotein. Elution profile of radiolabeled proteins from gelatin-Sepharose affinity column (A), Sephacryl S-300 molecular sieve column (B), and hydroxylapatite column (C). Pooled fractions from the three different column eluates, which contain the $M_r = 190,000$ glycoprotein, are designated I, II, and III, respectively.

**Fig. 4.** NaDodSO$_4$-polyacrylamide gel electrophoresis patterns of the $M_r = 190,000$ glycoprotein at various stages of purification. Samples were subjected to electrophoresis in 6% polyacrylamide gels in the absence (lanes 1-4) and presence (lanes 5-8) of 50 mM dithiothreitol (DTT) and stained with 0.25% Coomassie blue. Lanes 1 and 5, 20–50% ammonium sulfate fractionation; lanes 2 and 6, preparation following gelatin-Sepharose chromatography; lanes 3 and 7, preparation following Sephacryl S-300 chromatography; lanes 4 and 8, preparation following hydroxylapatite chromatography. The $M_r = 190,000$ glycoprotein (GP), fibronectin (FN), and bovine serum albumin (BSA) are identified.
Coomassie blue staining, although, on occasion, very low levels of contaminating protein were detectable by fluorescence autoradiography.

Endothelial cell medium pooled from 25 culture dishes (150 × 25 mm) provided approximately 0.3 mg of purified \( M_r = 190,000 \) glycoprotein with a final estimated recovery of 8% based on radioactivity (Table I). It became increasingly difficult to maintain high yields at the later purification steps. This problem was partially overcome by the addition of urea to the buffers used in the Sephacryl S-300 and hydroxylapatite columns and the use of siliconized glass tubes for the collection and storage of the protein. In spite of these precautions, handling of the purified \( M_r = 190,000 \) glycoprotein usually resulted in poor yields. This low recovery was due either to the nonspecific adhesion of the glycoprotein to surfaces or to the aggregation and precipitation of the glycoprotein from solution.

The amino acid composition of the purified \( M_r = 190,000 \) glycoprotein, shown in Table II, indicates that it contains relatively high levels of cysteine, aspartic acid (or asparagine), and glutamic acid (or glutamine). The amino acid composition clearly distinguished the \( M_r = 190,000 \) glycoprotein from \( \alpha_2 \)-macroglobulin.

A recent report by Doyle et al. (24) indicated that human umbilical vein endothelial cells produced a \( M_r = 145,000 \) glycoprotein that corresponded to thrombospondin, a glycoprotein released by platelets in response to thrombin. A comparison of the amino acid composition of the \( M_r = 190,000 \) glycoprotein with that of human thrombospondin indicated a high degree of similarity (Table II). This observation prompted us to purify thrombospondin from bovine platelets in order to compare the two glycoproteins more carefully. It was apparent that the major thrombin-released protein from bovine platelets (thrombospondin) co-migrated with the \( M_r = 190,000 \) glycoprotein, purified from BAE cell culture media, on NaDodSO\(_4\)-polyacrylamide gel electrophoresis (Fig. 6). In addition, a \( M_r = 160,000 \) glycoprotein (carbohydrate content detected by periodic acid-Schiff staining) which co-purified with thrombospondin was noted. Similar results were obtained when the migration of human platelet thrombospondin was compared with that of purified \( M_r = 190,000 \) glycoprotein (data not shown); however, the \( M_r = 160,000 \) glycoprotein was not detected in the thrombin-released material from human platelets. Purification of the thrombin-released material from bovine platelets indicated that both thrombospondin and the \( M_r = 160,000 \) glycoprotein eluted from the Sephacryl S-300 and hydroxylapatite columns at positions identical with that of the \( M_r = 190,000 \) glycoprotein secreted by bovine aortic endothelial cells. Lanes 4 and 8 of Fig. 6 illustrate that the combination of Sephacryl S-300 chromatography and hydroxylapatite chromatography provided a high degree of purification of both thrombospondin and the \( M_r = 160,000 \) glycoprotein.

Proteins solubilized from whole bovine platelets by NaDodSO\(_4\) buffer as well as proteins released by thrombin activation were subjected to electrophoresis on NaDodSO\(_4\)-polyacrylamide slab gels, together with purified \( M_r = 190,000 \) endothelial cell glycoprotein. These proteins were transferred to nitrocellulose paper and reacted with an antiserum to the \( M_r = 190,000 \) glycoprotein. As shown in Fig. 7, only thrombospondin homologues were detected in the bovine platelet preparations.

### Table I

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Glycoprotein*</th>
<th>Yield</th>
<th>Total protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>dpm</td>
<td>%</td>
<td>mg</td>
</tr>
<tr>
<td>Media*</td>
<td>1.1 × 10⁶</td>
<td>40.1</td>
<td>40.1</td>
</tr>
<tr>
<td>Ammonium sulfate (20-50%)</td>
<td>8.2 × 10⁷</td>
<td>75</td>
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<td>Gelatin-Sepharose eluate</td>
<td>6.4 × 10⁷</td>
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<td>Sephacryl S-300 pool</td>
<td>3.6 × 10⁷</td>
<td>33</td>
<td>2.4</td>
</tr>
<tr>
<td>Hydroxylapatite pool</td>
<td>8.4 × 10⁷</td>
<td>8</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* Percentage of the total disintegrations per minute in a particular sample which represented the \( M_r = 190,000 \) glycoprotein was determined by scanning the fluorography autoradiogram of the NaDodSO\(_4\)-polyacrylamide gel corresponding to that stage of purification (Fig. 5).

* Protein concentration was determined by the method of Lowry et al. (30).

### Table II

<table>
<thead>
<tr>
<th>Amino acid composition of the ( M_r = 190,000 ) glycoprotein compared with ( \alpha_2 )-macroglobulin and thrombospondin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values expressed as residues/1000. Tryptophan was not determined.</td>
</tr>
<tr>
<td>Bovine ( M_r = 190,000 ) glycoprotein*</td>
</tr>
<tr>
<td>Aspartic acid</td>
</tr>
<tr>
<td>Threonine</td>
</tr>
<tr>
<td>Serine</td>
</tr>
<tr>
<td>Glutamic acid</td>
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<tr>
<td>Proline</td>
</tr>
<tr>
<td>Glycine</td>
</tr>
<tr>
<td>Alanine</td>
</tr>
<tr>
<td>Cysteine</td>
</tr>
<tr>
<td>Valine</td>
</tr>
<tr>
<td>Methionine</td>
</tr>
<tr>
<td>Isoleucine</td>
</tr>
<tr>
<td>Leucine</td>
</tr>
<tr>
<td>Tyrosine</td>
</tr>
<tr>
<td>Phenylalanine</td>
</tr>
<tr>
<td>Histidine</td>
</tr>
<tr>
<td>Lysine</td>
</tr>
<tr>
<td>Arginine</td>
</tr>
</tbody>
</table>

* Amino acid analysis was performed as described under "Materials and Methods." Values represent the average of four separate analyses on two different preparations. The coefficient of variation for these determinations ranged from 1-10%.

* Lawler et al. (10).

* Swenson and Howard (19).
spondin and the $M_r = 160,000$ glycoprotein, among the complex mixture of proteins (see Fig. 6, lanes 6 and 7), reacted with the antibody. A cross-reaction with human thrombospondin was also observed (data not shown). Further evidence that the $M_r = 190,000$ glycoprotein and thrombospondin are identical or closely related was provided by a comparison of...
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yields decreased considerably. This loss appeared to be due to the strong tendency of the glycoprotein to adsorb to glass and plastic. These problems were partially circumvented by using siliconized glass and by adding urea to buffers. After lyophilization of pure M, = 190,000 glycoprotein from volatile buffers such as NH₄HCO₃, the glycoprotein was largely insoluble in aqueous buffers. Lyophilization of crude preparations of M, = 190,000 glycoprotein did not seem to affect its solubility appreciably.

We noted early in our studies that the M, = 190,000 glycoprotein migrated in close proximity to reduced α2-macroglobulin on NaDodSO₄-polyacrylamide gel electrophoresis. In fact, under certain conditions, the two proteins appeared to comigrate (Fig. 1). However, M, = 190,000 glycoprotein and α2-macroglobulin could be distinguished from each other on the basis of their mobility under nonreducing conditions (Fig. 1); while α2-macroglobulin migrated as a dimer under these conditions, the mobility of the M, = 190,000 glycoprotein indicated that it was a larger complex. Additional evidence that the M, = 190,000 glycoprotein and α2-macroglobulin are different proteins was obtained from the lack of immunological cross-reactivity, from comparison of two-dimensional peptide maps (data not shown), and from the significant differences in their amino acid compositions (Table II). Similar considerations distinguished the M, = 190,000 glycoprotein from fibronectin, another glycoprotein synthesized and secreted in large amounts by bovine aortic endothelial cells in culture. Lack of immunological cross-reactivity as well as significant differences in one-dimensional peptide maps of the two glycoproteins argued against the possibility that one was derived from the other. The amino acid composition of the M, = 190,000 glycoprotein was also clearly distinct from that of angiotensin-converting enzyme (25), another high molecular weight glycoprotein reported to be synthesized by endothelial cells in culture (26, 27).

Doyle et al. (24) have reported recently that human umbilical vein endothelial cells synthesize a M, = 145,000 glycoprotein which closely resembles platelet thrombospondin. Thrombospondin has also been referred to as glycoprotein G (28) and thrombin-sensitive protein (8). Several values for the apparent molecular weight of this protein, based on its mobility on NaDodSO₄ gels under reducing conditions, have been reported in the literature. Lawler et al. (10) reported that the protein is a disulfide-linked trimer with a subunit molecular weight of 142,000. Hagen et al. (9) determined a subunit molecular weight of 147,000 for the glycoprotein. In contrast, Phillips and Agin (29) and Baenziger et al. (8) reported molecular weights of 185,000 and 190,000, respectively, for the subunit of the disulfide-linked oligomeric protein. The similarity of the latter molecular weight estimates to that determined for the M, = 190,000 endothelial cell glycoprotein, together with the report by Doyle et al. (24), led us to examine the relationship between the M, = 190,000 glycoprotein and platelet thrombospondin.

We noted that the amino acid composition of the bovine M, = 190,000 glycoprotein is very similar to that reported for thrombospondin by Lawler et al. (10), albeit that the latter was obtained from human platelets. Further evidence that the M, = 190,000 glycoprotein and thrombospondin are closely related came from immunological and structural studies. Antibodies directed against the M, = 190,000 glycoprotein cross-reacted with bovine thrombospondin and a M, = 160,000 glycoprotein, also released by bovine platelets, as well as with human thrombospondin. The fact that the antibody reacted against both the M, = 190,000 and M, = 160,000 bands of thrombin-released material from bovine platelets suggests that the M, = 160,000 band may be a derivative of thrombospondin. However, if this is the case, processing or partial degradation of thrombospondin must have occurred prior to thrombin activation since both bands were present in whole platelets (Fig. 6) and seemed to disappear from the platelet pellet following thrombin-induced aggregation.

Two-dimensional peptide maps of the M, = 190,000 glycoprotein, bovine thrombospondin, and human thrombospondin were essentially indistinguishable, providing further evidence for their close similarity (Fig. 7). The map of the M, = 160,000 glycoprotein from bovine platelets shares many features with that of thrombospondin, indicating that it is either a derivative of thrombospondin or a closely related protein. The occurrence of a similar relationship for the human platelet protein could account for reports indicating lower molecular weights for thrombospondin (9, 10, 24). Although we have not observed the M, = 160,000 glycoprotein in human platelets, it is possible that differences in the methods of blood collection and platelet purification were responsible for the variation between the human and bovine preparations. We have noted that under certain conditions the glycoprotein purified from endothelial cell culture medium is partially converted to a form that migrates near the position of the M, = 160,000 protein observed in platelets.

Several important questions remain regarding thrombospondin. One relates to whether endothelial cells are uniquely responsible for the synthesis of this glycoprotein. In that case, platelets might acquire the protein from plasma in a manner similar to the uptake of fibrinogen. Alternatively, megakaryocytes may also synthesize the glycoprotein. A second question deals with the function of thrombospondin. Lactoperoxidase-mediated iodination of bovine aortic endothelial cells in culture indicated that the M, = 190,000 glycoprotein was associated with the surface of these cells. Since thrombospondin is also released from platelets upon thrombin-induced aggregation, this glycoprotein could be an integral component in the control of some aspect of the clotting process that involves platelet aggregation and thrombus formation. Thrombospondin could also function as a specific protease inhibitor of one of the enzymes of the coagulation cascade to delay clotting or as an activator to promote thrombus formation.

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REFERENCES


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