Human and Bovine Endothelial Cells Synthesize Membrane Proteins Similar to Human Platelet Glycoproteins IIb and IIIa*

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Human umbilical vein endothelial (HUVE) and bovine aortic endothelial (BAE) cells in culture were examined to determine whether membrane proteins similar to human platelet glycoproteins (GP) IIb and IIIa were present. The HUVE and BAE cells were either 125I-surface labeled or metabolically labeled. Triton X-100 lysates of labeled cells were immunoprecipitated with polyclonal antibodies prepared against purified human platelet GP IIb-IIIa complex. Two membrane proteins were detected on both HUVE (M, = 130,000 and 110,000) and BAE (M, = 135,000 and 105,000) cells, which were similar to human platelet GP IIb (M, = 125,000) and GP IIIa (M, = 108,000). The two membrane proteins from HUVE cells and the two from BAE cells cosedimented in sucrose gradients, indicating that they exist as a complex. Unlike the human platelet GP IIb-IIIa complex, the HUVE and BAE membrane protein complexes were not dissociated by chelation of Ca++. Platelet GP IIb and GP IIIa and the related membrane proteins on both HUVE and BAE cells showed similar changes in electrophoretic mobility upon disulfide reduction. These data demonstrate that human and bovine endothelial cells synthesize membrane proteins that have properties similar to the platelet membrane GP IIb-IIIa complex.

Endothelial cells provide a surface that is in contact with plasma proteins and cells normally found in circulation, as well as malignant cells that metastasize by hemogenous spread (1). Cultured endothelial cells have been shown to bind polymorphonuclear leukocytes (2), lymphocytes (3), monocytes (4), and stimulated platelets (5). Endothelial cells also bind proteins that are involved in coagulation (6) and agents that affect endothelial cell function, such as thrombin (7). However, with the exception of thrombomodulin (8), little is known about endothelial cell surface proteins and their possible receptor functions.

There are two reasons to expect that endothelial cell surface proteins may be similar to the cell surface proteins of platelets.

First, endothelial cells and platelets both have receptors for Factor Va (9, 10), Factor Xa (11, 12), thrombin (7, 13), and fibrinogen (14, 15). Second, endothelial cells synthesize and secrete several proteins found in platelet α-granules (e.g. von Willebrand factor (16), thrombospondin (17), and fibronectin (18)). The present report describes membrane proteins on human umbilical vein endothelial (HUVE) cells and bovine aortic endothelial (BAE) cells that are immunologically and biochemically similar to human platelet membrane glycoproteins (GP) IIb and IIIa.

**MATERIALS AND METHODS**

**Preparation of Antibodies—Antisera against the purified GP IIb-IIIa complex from human platelets** was produced in rabbits. The immunoglobulin G (IgG) fraction was purified by affinity chromatography using Protein A-Sepharose (Sigma) (19). Affinity purified GP IIb-IIIa antibody was obtained by incubating the GP IIb-IIIa antisera with nitrocellulose blots of GP IIa (20) and eluting the antibody from the washed nitrocellulose with 0.1 M glycine, pH 2.8.

**Cell Culture**—The HUVE cells were obtained by incubating umbilical veins for 10 min with Medium 199 (pH 7.4) that contained 1 mg/ml collagenase (Type I, Worthington) (21). The HUVE cells were cultured in Medium 199 that was supplemented with 20% fetal calf serum, 25 mM Hepes buffer (pH 7.4), 100 units/ml penicillin and streptomycin, 90 µg/ml porcine heparin (Sigma), and 100 mg/ml fibroblast growth factor (kindly provided by Dr. Denis Gospodarowicz, University of California, San Francisco, CA); 60-mm dishes (Falcon) coated with gelatin (Sigma) were used. Primary cultures were plated at 5 x 10^5 cells/dish, and the cells were grown to confluency at 37 °C in 5% CO_2 and 95% humidity. Cultured HUVE cells exhibited a typical cobblestone morphology (21). Primary and early passage HUVE cells were used for further study. The BAE cells, provided by Dr. George Rodgers (Gladstone Foundation Laboratories), were grown in Dulbecco’s modified Eagle’s medium (DMEM-H16) supplemented with 10% calf serum, and the cells between passages 5 and 10 were used. Human foreskin fibroblasts were grown in Dulbecco’s modified Eagle’s medium H21. Subcultures of HUVE, BAE, and fibroblast cells were obtained by detaching cells with 0.2 mg/ml EDTA and 0.5 mg/ml trypsin (4 min, 37 °C) and diluted 1:4 before replating. Tissue culture media and supplements were obtained from Gibco.

**Cell Surface Labeling—Confluent monolayers of HUVE and BAE cells were washed five times with serum-free medium and labeled with CuCl_2 (10^-4 M) at 37 °C and pH 7.2 (22).** The HUVE cells were again washed five times and lysed with Tris-buffered medium at room temperature in 1 ml of serum-free medium. Aliquots (10 µl) of 0.06% H_2O_2 were added every 1 min for 10 min (22). The cells were then washed and lysed with Tris-buffered saline (TBS) (20 mM Tris-HCl and 150 mM NaCl, pH 7.4) that contained 1% Triton X-100, 1 mM EDTA, and 1 mM phenylmethylsulfonyl fluoride. The lysates were centrifuged (15,000 x g) for 5 min.

**Platelet Isolation**—Platelets were isolated as described (23), surface labeled with ^125I (22), and lysed using TBS that contained 1% Triton X-100 and either 1 mM EDTA (37 °C and pH 5.5) or 1 mM CaCl_2 (4 °C and pH 7.2).

**Immunoprecipitation**—Cell lysates were centrifuged at 10,000 x g to remove cytoskeletal components (24).

The abbreviations used are: HUVE cells, human umbilical vein endothelial cells; BAE cells, bovine aortic endothelial cells; GP, glycoprotein; SDS, sodium dodecyl sulfate; IgG, immunoglobulin G; TBS, Tris-buffered saline; Hepes, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid.

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that contained 0.1% Triton X-100 and 1 mg/ml bovine serum albumin. Sodium dodecyl sulfate (SDS) was added to the lysates of the metabolically labeled cells (0.5%, final concentration). Glycoprotein IIb-IIIa antibody (20 μg), normal rabbit IgG (20 μg, Sigma), or the GP IIIa antibody (2 μg) was added, and the lysates were incubated overnight at 4 °C. A 25-μl aliquot of 10^9 Staphylococcus aureus Protein A (IgGorb, The Enzyme Center, Boston, MA) was added, and incubation was continued for 1 to 2 h at 4 °C. The Staphylococcus aureus Protein A was pelleted, washed twice with the TBS/Triton X-100/bovine serum albumin buffer, and heated to >80 °C in electrophoresis sample buffer.

**Electrophoresis and Western Blotting**—Sodium dodecyl sulfate-polyacrylamide (10%) gel electrophoresis was performed using the buffer system of Laemmli (25). Samples were prepared either with (reduced) or without (nonreduced) 2.5% β-mercaptoethanol (Sigma). Molecular weight standards were carbonic anhydrase (M_r = 29,000), ovalbumin (M_r = 43,000), bovine serum albumin (M_r = 66,000), phosphorylase b (M_r = 97,400), and β-galactosidase (M_r = 116,000) (Sigma). Gels were dried directly (121) or impregnated with ENHANCE (New England Nuclear) for fluorography (128S or 1H). A Bio-Rad Trans-Blot cell was used to transfer proteins from polyacrylamide gels to nitrocellulose (20). Autoradiography was performed using Kodak X-ray film at -70 °C.

**Sucrose Gradient Sedimentation**—Human platelets and HUVE and BAE cells were 125I surface labeled and lysed in TBS with 1% Triton X-100 that contained either 1 mM Ca^{2+} (4 °C, pH 7.2) or 1 mM EDTA (37 °C, pH 8.5). The EDTA cell lysates were incubated at 37 °C for either 5 min (platelets) or 30 min (HUVE and BAE cells) and then cooled to 4 °C until the gradients were begun (within 1 h). Linear 5 to 25% sucrose gradients were prepared in Beckman SW 55 tubes using TBS that contained 0.2% Triton X-100 and either 1 mM CaCl_2 (for Ca^{2+}-lysed cells) or 1 mM EDTA (for EDTA-lysed cells). Gradients were centrifuged at 4 °C for 7.5 h at 55,000 × g, drained from the bottom, and SDS-polyacrylamide gel electrophoresis was performed on the individual gradient fractions.

**RESULTS AND DISCUSSION**

The specificity of antibodies against GP IIb-IIIa and GP IIIa was determined by immunoprecipitation of Triton X-100-solubilized, 125I-labeled platelets (Fig. 1). Glycoproteins IIb, and IIIa were the predominant surface glycoproteins that were labeled by lactoperoxidase-catalyzed iodination of intact platelets (Fig. 1, lane 1). The GP IIb-IIIa antibody detected three bands (Fig. 1, lane 2); GP IIb (M_r = 125,000 reduced), GP IIIa (M_r = 108,000), and a band of M_r = 150,000. The M_r = 150,000 protein had a M_r = 140,000 in nonreduced gels (data not shown) and, therefore, appears to correspond to GP IIa (26, 27). When platelets were lysed using Ca^{2+}, the GP IIa antibody immunoprecipitated both GP IIb and GP IIIa (Fig. 1, lane 3), because the glycoproteins were present as a Ca^{2+}-dependent, heterodimer complex (28). However, when platelets were lysed using EDTA at 37 °C and pH 8.5, the GP IIIa antibody detected only GP IIIa (Fig. 1, lane 4), because, under these conditions, the GP IIb-IIIa complex was dissociated (28). The GP IIa antibody did not detect the M_r = 150,000 protein (GP IIa). Controls were negative (Fig. 1, lane 5). Thus, the “GP IIa-IIIa antibody” includes antibodies against GP IIa, while the affinity-purified “GP IIIa antibody” is specific for GP IIIa but also immunoprecipitates GP IIb if the two glycoproteins are complexed.

Fig. 2 shows that HUVE (lane 1) and BAE (lane 5) cells contained about 15 to 20 125I-surface-labeled proteins. Immunoprecipitation of the HUVE cell lysate with either the GP IIb-IIIa antibody (Fig. 2, lane 2) or the GP IIIa antibody (Fig. 2, lane 3) antibody detected bands of M_r = 130,000 and 110,000, which are molecular weights similar to those of human platelet GP IIb and GP IIIa. In addition, the GP IIb-IIIa antibody precipitated a third protein of M_r = 150,000, which may be related to human platelet GP IIa. A control immunoprecipitate was negative (Fig. 2, lane 4). Lysates of BAE cells that were immunoprecipitated with either the GP IIb-IIIa antibody (Fig. 2, lane 2) or the GP IIIa antibody (Fig. 2, lane 3) antibody contained labeled proteins of M_r = 135,000 and 105,000. These glycoproteins were labeled on apical monolayers, which indicates that they are on the apical surface of the cell. The GP IIa-IIIa protein was not detected in BAE cells. Table I compares the molecular weights of human and bovine platelet GP IIb and GP IIIa and the related membrane proteins of HUVE and BAE cells. These molecular weights were determined by comparing the immunoprecipitates of human platelets, HUVE, and BAE cells to protein standards all electrophoresed on the same gel. The characteristic shifts in electrophoretic mobility of human platelet GP IIb and GP IIIa upon disulfide reduction (26) also occurred with the corresponding membrane proteins of bovine platelets and endothelial cells.

Numerous proteins were labeled when HUVE cells were incubated with [125S]methionine (Fig. 3, lane 1). Three proteins, which corresponded in molecular weight to those from surface-labeled cells, were detected with the GP IIb-IIIa antibody (Fig. 3, lanes 2 and 5). Only the M_r = 130,000 and...
TABLE I

| Protein    | Human platelets | Human umbilical vein | Bovine platelets* | Bovine aorta |
|------------|-----------------|----------------------|-------------------|-------------|
| Nonreduced |                 |                      |                   |             |
| GP IIb     | 136,000         | 140,000              | 140,000           | 145,000     |
| GP IIIa    | 97,000          | 97,000               | 95,000            | 90,000      |
| Reduced    |                 |                      |                   |             |
| GP IIb0    | 125,000         | 130,000              | 125,000           | 135,000     |
| GP IIIa0   | 23,000          | ~25,000              | NDb               | NDb         |
| GP IIIa    | 108,000         | 110,000              | 100,000           | 105,000     |

* Determined by 125I cell surface labeling and immunoprecipitation with the GP IIb-IIIa antibody.

** ND, not detected.

110,000 proteins were detected with the GP IIIa antibody, and these proteins were not precipitated in controls (Fig. 3, lanes 4 and 6). Immunoprecipitates from [35S]methionine-labeled HAE cells (Fig. 3, lane 7) contained two labeled proteins with molecular weights identical to those of the surface-labeled proteins shown in Fig. 2. Control immunoprecipitates of HAE cells were negative (Fig. 3, lane 8). [35S]Methionine-labeled human fibroblasts were also negative (data not shown). This experiment demonstrates that endothelial cells synthesize the membrane proteins that are related to platelet GP IIb, GP IIIa, and GP IIa.

The GP IIIa antibody immunoprecipitated the two HUVE protein and the two BAE proteins, suggesting that these proteins were complexed. Sucrose gradient sedimentation was used to further examine this possibility. Glycoproteins IIb and IIIa from 125I-labeled platelets lysed with Ca2+-Triton X-100 co-sedimented as a complex (Fig. 4A). Lysates of 125I-labeled HUVE cells prepared with Ca2+-Triton X-100 had two proteins of Mr = 130,000 and 110,000 that co-sedimented with a sedimentation coefficient similar to that of the platelet GP IIb-IIIa complex (Fig. 4C). To positively identify these two labeled bands, they were immunoprecipitated from the individual sucrose gradient fractions with the GP IIIa antibody (Fig. 4E). In this experiment, an additional protein that had a molecular weight similar to that of human platelet GP IIb (26) was detected. This protein was not detected in earlier experiments (Figs. 2 and 3) because these gels used slightly lower percentages of acrylamide. The sedimentation of these HUVE proteins did not change when lysates were prepared using EDTA at 37 °C and pH 8.5 (data not shown). Thus, the complex of the HUVE GP IIb-IIIa-like proteins is not Ca2+-dependent. The Mr = 135,000 and 105,000 BAE proteins also co-sedimented with a sedimentation coefficient similar to that of the human platelet GP IIb-IIIa complex (Fig. 4D) and were selectively immunoprecipitated with the GP IIIa antibody (Fig. 4F). In addition, bovine platelet GP IIb and GP IIIa co-sedimented as a complex that could not be dissociated by Ca2+ chelation (data not shown). Thus, complex formation appears to be a common property of human and bovine platelet GP IIb-IIIa and the related endothelial proteins, but only human platelets have a complex that is Ca2+-dependent. These sucrose gradients also demonstrate that the GP IIb- and GP IIIa-like components are major endothelial cell surface proteins.

Recently, Thiagarajan et al. (29) described a human platelet GP IIIa-related membrane protein (Mr ~ 110,000) in HUVE cells in culture. These experiments used monoclonal antibodies against human platelet GP IIIa. It is surprising that these
authors did not detect the presence of a GP IIb-related protein, because the data in the present report indicate that these HUVE membrane proteins are complexed. Thus, antibodies that bind to either GP IIb or GP IIIa should detect both proteins. An additional difference is that Thiagarajan et al. (29) did not detect a change in electrophoretic mobility of the HUVE GP IIIa-like protein upon reduction of the disulfide bonds. These discrepancies could be due to differences in either (i) the number of passages of the cells in culture or (ii) the cell surface labeling methods.

The presence of endothelial cell membrane proteins that are related to platelet membrane proteins may be significant for two reasons. First, the platelet GP IIb-IIIa complex is a receptor for fibrinogen, fibronectin, and von Willebrand factor (30, 31). The demonstration that endothelial cells have a similar membrane protein complex suggests that endothelial cells may also have specific receptors for these three proteins. Indeed, Dejana et al. (14) have demonstrated specific binding of fibrinogen to HUVE cells. Second, because the platelet GP IIb-IIIa complex serves to mediate platelet aggregation (31), the related proteins on endothelial cells could serve as attachment sites for circulating cells. We are currently examining endothelial cells for the presence of additional platelet membrane proteins and other cells of the vessel wall for the presence of GP IIb- and GP IIIa-like proteins.

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