Platelet Glycoprotein IIb
Chromosomal Localization and Tissue Expression

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Abstract

The GPIIb-IIIa complex functions as a receptor for cytoadhesive proteins on the platelet surface. Both GPIIb and GPIIIa are synthesized by a human erythroleukemia (HEL) cell line. We isolated several cDNA clones by screening a HEL cell cDNA library with an oligonucleotide derived from amino acid sequence of GPIIb. Nucleotide and amino acid sequences were determined from 703 bp of one of these clones. Amino acid sequence of purified platelet GPIIb peptides confirmed the identity of the clone. The cDNA encodes the carboxyl terminus of the large (α) subunit of GPIIb and all of the smaller (β) subunit of GPIIb. By hybridizing the cDNA directly to chromosomes separated by dual laser chromosome sorting, the gene for GPIIb was mapped to chromosome 17. Northern blot analysis showed a $\sim$ 3.4-kb GPIIb mRNA in HEL cells. We also compared the amino acid sequences determined from eight additional platelet GPIIb peptides with the derived amino acids from a published HEL cell GPIIb cDNA, and the platelet and HEL cell proteins appear to be the same. Despite previous reports that vascular endothelial cells and monocytes contain GPIIb, no GPIIb mRNA was observed in either type of cell. Thus, GPIIb appears to be specific for the platelet-megakaryocyte membrane and is distinct from the α subunits of the adhesion receptors in other normal tissues.

Introduction

A critical step in maintaining normal hemostasis is the aggregation of platelets during the formation of blood clots. Failure of platelets to aggregate results in bleeding, as is seen in the hereditary disorder, Glanzmann's thrombasthenia (1). The platelets of these patients are deficient in membrane GPIIb and GPIIIa (2). GPIIb and GPIIIa mediate platelet aggregation by acting as the receptor for fibrinogen, an adhesion molecule

that binds platelets to one another in the blood clot (3). As determined by SDS-PAGE, unreduced GPIIb has an apparent M_r of 142,000 (4). Electrophoresis of the reduced protein reveals two disulfide-linked subunits; GPIIb$_\alpha$ (M_r of 123,000) and GPIIb$_\beta$ (M_r of 22,000) (4) that are synthesized as a single-chain precursor (5). GPIIIa is a single-chain glycoprotein. GPIIb and GPIIIa are associated as a noncovalent, calcium-dependent heterodimer in the platelet membrane (for review, see reference 1).

Recent evidence suggests that the GPIIb-IIIa complex belongs to a class of receptors that bind cell adhesion molecules (for review, see reference 6). These receptors share a common heterodimeric structure with α and β subunits. Platelet GPIIb and GPIIIa correspond to the α and β subunit, respectively (not to be confused with GPIIb$_\alpha$ and GPIIb$_\beta$, the subunits of reduced mature platelet GPIIb). GPIIb-IIIa is able to bind not only fibrinogen (3), but other cytoadhesive proteins, such as fibronectin (7), vitronectin (8), and von Willebrand factor (9). In contrast, Pytela et al. observed that when extracted from human placenta, the receptor for fibronectin binds only fibronectin, and that the vitronectin receptor binds only to vitronectin (8). All of these adhesion molecules contain the cell attachment sequence Arg-Gly-Asp (10), but the factors that confer ligand specificity (i.e., fibrinogen binds to GPIIb-IIIa but not to the fibronectin receptor [8]) are unknown.

Study of the genes encoding platelet proteins is particularly difficult for two reasons: (i) platelets are anucleate and synthesize little or no protein (11), and (ii) megakaryocytes, the precursor cells of platelets, comprise only 0.1% of all cells in the bone marrow and are therefore difficult to purify quantitatively. The human erythroleukemia (HEL)[1] cell line possesses some features of megakaryocytes (12, 13). Specific polyclonal antibodies raised against reduced or nonreduced GPIIb or GPIIIa have been shown to react with HEL cell proteins of comparable molecular weight on Western blots (5, 12). At least seven different monoclonal antibodies against platelet GPIIb, GPIIIa, or the GPIIb-IIIa complex react with intact HEL cells (12–15). Therefore, HEL cells represent a good model for analysis of the genes for GPIIb and GPIIIa.

We have constructed a cDNA library in the λgt10 vector using HEL cell RNA, and have cloned and sequenced a partial cDNA for GPIIb. Poncz et al. have recently published the cDNA for the entire coding region of GPIIb, also derived from HEL cell RNA (16). We report here that the gene for GPIIb is located on chromosome 17. Also, Northern blot analysis re-

1. Abbreviations used in this paper: HEL, human erythroleukemia; HUVE, human umbilical vein endothelial.

veals the absence of GPIIb mRNA in human umbilical vein endothelial (HUVE) cells and peripheral blood monocytes, cells in which GPIIb-like proteins have been previously reported. We also provide additional platelet amino acid sequence confirming the equivalence of HEL cell and platelet GPIIb.

Methods

GPIIb amino acid sequence determination. GPIIb was isolated as described (5). Methods of amino acid determination will be described in detail in another manuscript (manuscript in preparation). Briefly, purified reduced–alkylated GPIIb was digested with trypsin and separated by reverse-phase HPLC. Amino acid sequencing from the NH_2 terminus of intact IIb_α and the resultant peptides was performed using a gas phase protein sequencer (model 470 A; Applied Biosystems, Inc., Foster, City, CA). Several attempts to sequence the NH_2 terminus of IIB_β proved unsuccessful. Therefore, IIb_β was cleaved by cyanogen bromide and subjected to sequencing.

Oligonucleotide synthesis. Using the amino acid sequence of $GPIIb_\beta$, a 48-bp oligonucleotide was designed according to Lathe (17) and synthesized by the phosphotriester method (Oligonucleotide Chemistry Systems Laboratories, Denton, TX).

Tissues. Human peripheral blood monocytes were isolated from a normal donor platelet pheresis residue bag, as described (18). After separation, Wright's stain and esterase stain showed 96% monocytes, 4% lymphocytes, and no visible platelets. HUVE cells were isolated from freshly prepared human umbilical cords by collagenase digestion and cultured with serial passages as described (19).

RNA preparation. Total RNA was extracted from various cell lines and tissues by the guanidinium thioisocyanate method (20). $Poly(A)^+$ RNA was selected by elution from an oligo(dT)–cellulose column (21). For construction of the cDNA library, HEL cell $poly(A)^+$ RNA was fractionated on a 15–30% sucrose gradient, and then subjected to a second purification over an oligo(dT)–cellulose column. The various $poly(A)^+$ RNA fractions were translated in vitro and the resulting proteins immunoprecipitated and electrophoresed to determine the fraction most enriched for GPIIb mRNA. Fractions corresponding to $poly(A)^+$ RNAs of 3 kb and larger were pooled.

cDNA library construction. 4 μg of size-selected $poly(A)^+$ RNA was used to synthesize the oligo(dT)–primed first strand of cDNA (22). Second strand was made using RNase H and DNA polymerase I (23). The cDNA was made blunt-ended, treated with Eco RI methylase, ligated to Eco RI linkers (Pharmacia Fine Chemicals, Piscataway, NJ) and digested with Eco RI (22). This cDNA was fractionated on a 1% agarose gel to size-select for cDNAs > 0.9 kb and to remove free linkers. cDNAs were eluted from gel slices, and then ligated into the λgt10 vector and packaged (both λgt10 vector and packaging extract from Stratagene Cloning Systems, San Diego, CA).

Screening of the λgt10 HEL cell library. The $GPIIb_\beta$ 48-mer was end-labeled with $\gamma[^{32}P]ATP$, and 2×10^6 cpm probe per milliliter hybridization solution was used to screen 700,000 recombinants. Hybridization conditions were: 5× standard saline citrate (SSC) (1× SSC is 150 mM NaCl, 15 mM sodium citrate)/5× Denhardt's solution (Denhardt's is 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% BSA)/10% formamide at 37°C for 12 h. Filters were washed in 2× SSC at 22°C, then in 0.3× SSC/0.1% SDS at 51°C. Phage DNA was prepared and digested with Eco RI. The sizes of the cDNA inserts ranged from 720 to 2,000 bp. The Eco RI insert from one of the positive clones, λH3.1, was ligated into the pBS plasmid vector (Stratagene Cloning Systems).

Nucleotide sequencing. The Eco RI insert of λH3.1 was ligated into M13mp19 and the nucleotide sequence was determined using the dideoxynucleotide chain termination method (24).

Chromosomal localization of the GPIIb gene. The chromosomal localization of the GPIIb gene was analyzed by hybridization of the cDNA directly to the DNA of chromosomes resolved by dual laser chromosome sorting (25).

cDNA probes. Northern blots were probed with the ^{32}P-labeled 0.7 kb Eco RI insert of λH3.1 and with a 2.2-kb Eco RI fragment from the 5' end of the cDNA for GPIIIa (manuscript in preparation). This latter cDNA was obtained from the HEL cell cDNA library described above and is 99% homologous to that obtained by Fitzgerald et al. from a HUVE cell cDNA library (26). Additional probes used as positive controls were a 404-bp Eco RI fragment of the 5' end of the von Willebrand factor cDNA (19) and the 3' 1.8 kb Eco RI fragment of the β subunit of the leukocyte adhesion proteins (27).

Northern blot analysis. $Poly(A)^+$ RNA was electrophoresed on a 1% agarose 2.2 M formaldehyde gel and blotted to nitrocellulose or nylon filters. Nitrocellulose filter hybridization was performed with 2×10^6 cpm/ml probe in 50% formamide/1× Denhardt's solution/3× SSC/50 mM $NaPO_4$ (pH 7.0)/200 μg/ml salmon sperm DNA/10% dextran sulfate at 42°C for 12 h. Filters were washed in 0.1× SSC/0.1% SDS at 55°C. Nylon filters were hybridized with 2×10^6 cpm/ml probe in 7% SDS/0.5 M $NaPO_4$ (pH 7.2)/10% dextran sulfate at 65°C and washed in 2× SSC/1% SDS at 65°C.

Results

A cDNA library containing 2.6×10^6 recombinants was constructed from HEL cell $poly(A)^+$ RNA. 700,000 recombinants were screened at high plating density and 28 clones were identified that reacted with a $GPIIb_\beta$ synthetic oligonucleotide. Only twelve were pursued on secondary screening, and seven of these clones were positive on secondary and tertiary screening. To confirm the authenticity of one clone, λH3.1, its nucleotide sequence was determined (Fig. 1). When translated, the open reading frame corresponds to 172 amino acids. The derived sequence was compared with 11 residues of a $GPIIb_\beta$ peptide (Table I, peptide 6) obtained by tryptic digestion; all 11 matched (Fig. 1, amino acids 111–121). We also compared the derived sequence with 19 residues of a $GPIIb_\beta$ peptide obtained by cleavage with cyanogen bromide (Table I, peptide $\beta CNBr$); 17 amino acids matched (Fig. 1, amino acids 154–170) and the first and last residues were ambiguous. In both discrepant residues, a minor HPLC signal was identical to the nucleotide-derived amino acid. Hiraiwa et al. (28) sequenced two GPIIb peptides, one of which matched 12 of our derived amino acids, 161–172 (Fig. 1). Because their sequence overlapped all but one of our $GPIIb_\beta$ peptide residues, it was not indicated in Fig. 1. Moreover, all 15 residues of the $GPIIb_\beta$ NH_2 terminus published by Charo et al. (29) were identical with a segment of our predicted sequence (Fig. 1, amino acids 36–50). We conclude that the λH3.1 cDNA includes the entire sequence for $GPIIb_\beta$. The cDNA also codes for 35 amino acids that precede the NH_2 terminus of $GPIIb_\beta$ and presumably represents the COOH-terminal end of $GPIIb_\alpha$.

We compared both our cDNA nucleotide sequence and platelet GPIIb peptide sequences with the data of Poncz et al. (16). All 703 nucleotides of our λH3.1 clone matched perfectly with the 3' end of their cDNA (comparison not shown). Table I lists the 10 platelet GPIIb peptides that we sequenced. Peptides 6 and "$\beta CNBr$" are contained in $GPIIb_\beta$ and shown in Fig. 1. Of the remaining 8 peptides, 153 residues were assigned. When compared with the published amino acid sequence derived from the HEL cell GPIIb cDNA (16), 151 matched (comparison not shown).

The chromosomal location of the GPIIb gene was analyzed by hybridization of the ^{32}P-labeled λH3.1 Eco RI restriction fragment directly to the DNA of chromosomes resolved by

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  1        Val  Asp  Trp  Gly  Leu  Pro  Ile  Pro  Ser  Pro  Ser  Pro
  1    AG  GTG  GAC  TGG  GGG  CTG  CCC  ATC  CCC  AGC  CCC  TCC  CCC

 13    Ile  His  Pro  Ala  His  His  Lys  Arg  Asp  Arg  Arg  Gln  Ile
 39    ATT  CAC  CCG  GCC  CAT  CAC  AAG  CGG  GAT  CGC  AGA  CAG  ATC
                                                                ↓
 26    Phe  Leu  Pro  Glu  Pro  Glu  Gln  Pro  Ser  Arg  Leu  Gln  Asp
 78    TTC  CTG  CCA  GAG  CCC  GAG  CAG  CCC  TCG  AGG  CTT  CAG  GAT

 39    Pro  Val  Leu  Val  Ser  Cys  Asp  Ser  Ala  Pro  Cys  Thr  Val
117    CCA  GTT  CTC  GTA  AGC  TGC  GAC  TCG  GCG  CCC  TGT  ACT  GTG

 52    Val  Gln  Cys  Asp  Leu  Gln  Glu  Met  Ala  Arg  Gly  Gln  Arg
156    GTG  CAG  TGT  GAC  CTG  CAG  GAG  ATG  GCG  CGC  GGG  CAG  CGG

 65    Ala  Met  Val  Thr  Val  Leu  Ala  Phe  Leu  Trp  Leu  Pro  Ser
195    GCC  ATG  GTC  ACG  GTG  CTG  GCC  TTC  CTG  TGG  CTG  CCC  AGC

 78    Leu  Tyr  Gln  Arg  Pro  Leu  Asp  Gln  Phe  Val  Leu  Gln  Ser
234    CTC  TAC  CAG  AGG  CCT  CTG  GAT  CAG  TTT  GTG  CTG  CAG  TCG

 91    His  Ala  Trp  Phe  Asn  Val  Ser  Ser  Leu  Pro  Tyr  Ala  Val
273    CAC  GCA  TGG  TTC  AAC  GTG  TCC  TCC  CTC  CCC  TAT  GCG  GTG

104    Pro  Pro  Leu  Ser  Leu  Pro  Arg  Gly  Glu  Ala  Gln  Val  Trp
312    CCC  CCG  CTC  AGC  CTG  CCC  CGA  GGG  GAA  GCT  CAG  GTG  TGG

117    Thr  Gln  Leu  Leu  Arg  Ala  Leu  Glu  Glu  Arg  Ala  Ile  Pro
351    ACA  CAG  CTG  CTC  CGG  GCC  TTG  GAG  GAG  AGG  GCC  ATT  CCA

130    Ile  Trp  Trp  Val  Leu  Val  Gly  Val  Leu  Gly  Gly  Leu  Leu
390    ATC  TGG  TGG  GTG  CTG  GTG  GGT  GTG  CTG  GGT  GGC  CTG  CTG

143    Leu  Leu  Thr  Ile  Leu  Val  Leu  Ala  Met  Trp  Lys  Val  Gly
429    CTG  CTC  ACC  ATC  CTG  GTC  CTG  GCC  ATG  TGG  AAG  GTC  GGC

156    Phe  Phe  Lys  Arg  Asn  Arg  Pro  Pro  Leu  Glu  Glu  Asp  Asp
468    TTC  TTC  AAG  CGG  AAC  CGG  CCA  CCC  CTG  GAA  GAA  GAT  GAT

169    Glu  Glu  Gly  Glu  Stop
507    GAA  GAG  GGG  GAG  TGA  TGGTGCAGCCTACACTATTCTAGCAGGAGGGTTG

556    GGCGTGCTACCTGCACCGCCCCTTCTCCAACAAGTTGCCTCCAAGCTTTGGGTTGG
612    AGCTGTTCCATTGGGTCCTCTTGGTGTGCGTTTCCCTCCCAACAGAGCTGGGCTACC
668    CCCCCTCCTGCTGCCTAATAAAGAGACTGAGCCCTGAAAAAAAAAAAA(n)
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Figure 1. Derived amino acid and nucleotide sequence of partial HEL cell GPIIb cDNA. The λH3.1 clone was subcloned into M13mp19 and complete sequence was obtained in both orientations. Numbering begins at the 5′ end of the λH3.1 clone. Identical platelet peptide sequences are indicated by double-underlined regions. Underline breaks indicate ambiguities in peptide sequencing. The boxed region is the putative hydrophobic transmembrane segment (16). The single underline at nucleotide 684 represents the polyadenylation signal. The potential N-linked glycosylation site (*) and the IIb$_\alpha$-IIb$_\beta$ cleavage site (↓) are indicated.

dual laser chromosome sorting (Fig. 2). The gene for GPIIb mapped uniquely to chromosome 17. Not shown are chromosomes 9–12. In a second experiment, the λH3.1 clone did not hybridize to chromosomes 9–12, but did bind to chromosome 17.

To study tissue expression, we used the λH3.1 cDNA and a GPIIIa cDNA fragment to probe for mRNA expression in HEL cells, HUVE cells, and normal human circulating monocytes. HEL cells contain a single ~ 3.4-kb species of GPIIb mRNA (Fig. 3, lane 1) and a ~ 6-kb species of GPIIIa RNA (Fig. 3, lane 2). The faint signal at ~ 3-kb (Fig. 3, lane 2) has been a consistent observation and may represent a spliced GPIIIa message. GPIIb message was not detected in mRNA from either HUVE cells or monocytes (Fig. 3, lanes 3 and 6, respectively). When endothelial mRNA was probed with GPIIb cDNA under less stringent conditions (same as in Methods, except hybridization performed at 37°C and washing performed in 2× SSC/0.1% SDS), no GPIIb message was detected (data not shown). However, when probed with GPIIIa cDNA, a single ~ 6-kb message was observed in mRNA from HUVE cells, but none in the monocytes (Fig. 3, lanes 4 and 7,

respectively). As positive controls, a cDNA probe for von Willebrand factor (Fig. 3, lane 5) and for the β subunit of the leukocyte adhesion proteins (Fig. 3, lane 8) identified the appropriate ~ 8.5 kb (30) and ~ 3.0 kb (27) mRNAs, respectively, in endothelial cells and monocytes. Note that when the filter used in Fig. 3, lane 3 was probed with the GPIIIa cDNA, the ~ 6-kb mRNA was seen (data not shown), and that Fig. 3, lanes 6, 7, and 8 represent the same filter hybridized in three separate experiments. Northern blot analysis indicated ~ 20-fold less GPIIb mRNA in K562 cells than in HEL cells, and absence of GPIIb mRNA in reticulocytes (data not shown).

Discussion

We have obtained a partial cDNA clone coding for the COOH-terminal portion of GPIIb. There was perfect identity between the derived amino acids of this cDNA and 43 amino acids from platelet GPIIb$_\beta$. The 703 nucleotides of our cDNA clone are identical to the 3′ end of the cDNA published by Poncz et al. (16). Additionally, we found 151 of 153 amino acids from 8 platelet GPIIb peptides matched the recently

Table I. Amino Acid Sequence from Platelet GPIIb Peptides

Peptide	Sequence
	D
αNH₂ terminus	L N L D P V Q L T F Y A G P (X) G S Q F G F S L T F H K (X) S H G (R) V A (I) V (V) G
1	N V G S Q T L Q T F K
2	I Y V (E) N D F S W D K R
3	V Y L F L Q P R
4	F (X) S A I A P L G D L D (X) D G Y N (X) I A V A A P Y G G P S G R
5	(A) L G P S Q E E T G G V F L (X) P W R
6	G E A Q V W T Q L L R
7	G P H A L G A P S L L L T G T Q L Y G R
8	(X) R P S Q V L D S P F D T G S A F G F S L R
βCNBr	(S) K V G F F K R N R P P L E E D (D) (E) (Y)

Note row 8 has **P** above it.

Amino acid sequences of the NH₂ termini of GPIIb$_\alpha$, tryptic peptides of GPIIb, and the cyanogen bromide peptide (βCNBR) of GPIIb$_\beta$. Amino acid residues are given in the single-letter code. Tryptic peptides were identified by assigning a number to the peak on the chromatographic profile. Tentatively assigned residues are in parentheses; undetermined residues are indicated by "X". The published cDNA-derived amino acids (16) at variance with our residues are indicated above our peptides and in bold lettering.

published sequence of HEL cell GPIIb (16). When considered in light of immunologic and biochemical evidence (5, 12–15), the data strongly suggest that platelet and HEL cell GPIIb are the same protein.

Northern blot analysis of HEL cell poly(A)⁺ RNA reveals that the message for GPIIb is ~ 3.4 kb. This is somewhat smaller than the 4.1-kb reported by Poncz et al. (16). The discrepancy is likely due to the different denaturing gel systems used and/or the different molecular weight markers employed. Once the 5' untranslated region has been cloned, the true mRNA size can be determined.

Several reports have indicated that human endothelial cells synthesize a complex related to GPIIb-IIIa (31–34). When cultured HUVE cells were surface radiolabeled, solubilized, and immunoprecipitated with anti-GPIIIa antisera, an α-β heterodimeric complex was observed (31–34). Based on immunologic evidence (35) and comparisons of partial peptide sequence from GPIIIa with the sequence from an endothelial cell cDNA (26), it appears that platelet GPIIIa and the endothelial β subunit are identical. However, direct immunological evidence that the α subunit of HUVE cells is platelet GPIIb has never been obtained. Moreover, our present work shows ab-

sence of GPIIb mRNA from HUVE cells. Also, we have been unable to detect an immunologically related GPIIb molecule in HUVE cells by Western blotting using a polyclonal anti-GPIIb antibody (unpublished observation). These data indicate that once the HUVE cell adhesion receptor complex is dissociated by SDS-PAGE, the α subunit is not recognized by anti-GPIIb antisera. Our findings are in disagreement with an

Figure 3. Identification of GPIIb and GPIIIa mRNA by Northern blot analysis Poly(A)⁺ RNA from HEL cells (10 μg, lanes *1* and *2*), HUVE cells (3 μg, lane *3;* 2 μg, lane *4;* 3 μg, lane *5*), and normal human circulating monocytes (1 μg, lanes *6, 7,* and *8*) was electrophoresed and transferred to nitrocellulose. These filters were probed with ³²P-labeled cDNA for GPIIb (lanes *1, 3,* and *6*), GPIIIa (lanes *2, 4,* and *7*), von Willebrand factor (lane *5*), and the β subunit of the leukocyte adhesion proteins (lane *8*). Lanes *1* and *2* exposed at 22°C without intensifying screens for 12 h. Lanes *3–8* exposed at −70°C with intensifying screens for 12 h (lanes *3–5* and *8*) or 4 d (lanes *6* and *7*). 28S and 18S ribosomal markers are indicated by the upper and lower arrows, respectively, in each panel.
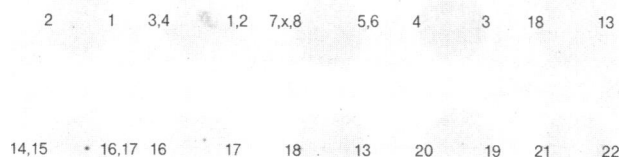

Figure 2. Autoradiogram of dot-blot filters of sorted chromosomes. ³²P-radiolabeled cDNA (0.72 kb restriction fragment) was hybridized to the DNA of chromosomes resolved by dual laser chromosome sorting and bound to nitrocellulose. Numbers refer to chromosome(s) isolated.
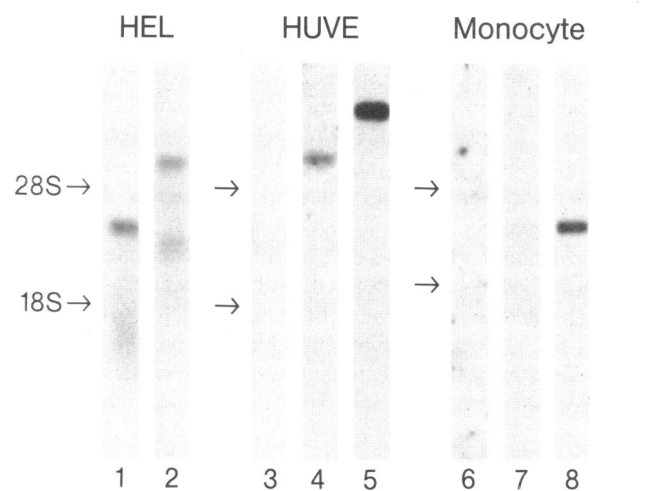
earlier report in which antisera apparently specific for GPIIb$_\alpha$ immunoprecipitates the HUVE cell α-β heterodimeric complex (29). An explanation for this disagreement would be that the anti-GPIIb$_\alpha$ antisera has minor reactivity with GPIIIa. Thus, in contrast to GPIIIa, our results indicate that the α subunit of the HUVE cell heterodimer is distinct from platelet GPIIb. The term "GPIIb" should not be used to describe the α subunit of HUVE cells.

The identity of the α subunit of the endothelial cell adhesion receptor is not known. When we probed endothelial mRNA with the λH3.1 cDNA under nonstringent conditions, no homologous mRNA was identified. The adhesion receptor on endothelial cells may be analogous to the vitronectin receptor, in which the β subunit is likely to be identical to platelet GPIIIa and the α subunit different from GPIIb (36).

There has been speculation that the β subunits of other cell adhesion receptors share more homologies than the α subunits (37). Since GPIIb-IIIa binds additional ligands not bound by other adhesion receptors (7), our findings suggest that GPIIb is likely to be important in conferring receptor–ligand specificity.

Several comments regarding monocyte expression of GPIIb can be made based on the results of our Northern blot analysis. Evidence for (38–40) and against (41, 42) the presence of GPIIb and GPIIIa in monocytes has been presented. Our data reveals no GPIIb or GPIIIa mRNA in monocytes. Because we analyzed only 1 μg of poly(A)$^+$ monocyte RNA, we cannot rule out very low levels of expression. However, probing the same filter under identical hybridization conditions, we were able to detect both the Factor XIII a-chain, a cytoplasmic protein, (18) and the β subunit of the leukocyte adhesion proteins (Fig. 3, lane 8), a membrane cytoadhesion protein similar to GPIIIa. Therefore, it is unlikely that monocytes contain either GPIIb or GPIIIa.

We have mapped the gene for GPIIb to chromosome 17. We are currently conducting experiments to localize the gene for GPIIIa. These results will have implications with regard to the genetic mechanism for Glanzmann's thrombasthenia. If the gene for GPIIIa is on chromosome 17, then either a gene deletion or a defect in a regulatory element controlling transcription of both genes could explain why GPIIb and GPIIIa are absent or decreased in parallel in the platelets of patients with this disease.

The ligand binding sites on the GPIIb-IIIa complex and the structural features required for the subunit assembly have not been characterized. Cloning of the cDNA for GPIIb and GPIIIa will allow experiments in which the protein products can be selectively altered so that answers to these questions can be obtained.

Acknowledgments

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