Proc. Natl. Acad. Sci. USA
Vol. 84, pp. 5615–5619, August 1987
Biochemistry

Cloning of the α chain of human platelet glycoprotein Ib: A transmembrane protein with homology to leucine-rich α_2-glycoprotein

(cDNA cloning/cDNA sequence analysis/platelet adhesion)

JOSE A. LOPEZ*†, DOMINIC W. CHUNG*, KAZUO FUJIKAWA*, FREDERICK S. HAGEN‡, THALIA PAPAYANNOPOULOU†, AND GERALD J. ROTH†§¶

Departments of *Biochemistry and †Medicine, University of Washington, §Veterans Administration Medical Center, and ‡ZymoGenetics, Inc., Seattle, WA 98195

Communicated by Earl W. Davie, April 27, 1987

ABSTRACT **Glycoprotein Ib is a surface membrane glycoprotein of platelets that functions as a receptor for von Willebrand factor. It is a heterodimer composed of an α and a β chain linked by a disulfide bond(s). A phage λgt11 cDNA expression library prepared from mRNA from a human erythroleukemia cell line, HEL, was screened using an affinity-purified antibody to the glycocalicin portion of the α chain of glycoprotein Ib. Eleven positive clones were isolated and plaque-purified. The largest cDNA insert was 2420 nucleotides in length and coded for a leader sequence of 16 amino acids, a mature protein of 610 amino acids, and a stop codon. It also contained 42 nucleotides of 5′ noncoding sequence and 497 nucleotides of 3′ noncoding sequence, including a poly(A) tail. The amino acid sequence of the α chain of GPIb predicted from the cDNA agreed completely with the sequence of 156 amino acids that was determined by Edman degradation of peptides isolated from human platelet glycocalicin after digestion with trypsin or *Staphylococcus aureus* V8 protease. The extracytoplasmic domain of the α subunit of GPIb contains several noteworthy structural features, including a region of seven tandem repeats of 24 amino acids that are homologous with those present in leucine-rich α_2-glycoprotein. The extracytoplasmic domain also contains two hydrophilic regions, one rich in charged amino acids and a second rich in serine and threonine residues. The region rich in serine and threonine includes five repeats of nine amino acids as well as the majority of the O-linked carbohydrate sites present in the molecule. The extracytoplasmic domain is followed by a potential transmembrane segment of approximately 29 amino acids and a potential intracellular domain of approximately 100 amino acids located at the carboxyl end of the molecule.**

The initial hemostatic response to blood vessel injury is platelet plug formation. One of the critical events in this reaction is the adhesion of platelets to the subendothelium. Adhesion requires the binding of platelet membrane glycoprotein Ib (GPIb) to von Willebrand factor (vWF) following the binding of vWF to the subendothelial matrix.

GPIb is present on the platelet plasma membrane as a heterodimer composed of an α chain (GPIbα) and a β chain (GPIbβ) linked by a disulfide bond(s). The heterodimer has an apparent molecular mass of 160 kDa. Following reduction, GPIbα and GPIbβ migrate with molecular masses of 145 kDa and 22 kDa, respectively, as determined by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (1). Both chains appear to be integral membrane glycoproteins containing hydrophobic transmembrane domains (2). During platelet lysis in the absence of protease inhibitors, GPIbα is cleaved by an endogenous calcium-dependent sulfhydryl protease (3), which gives rise to glycocalicin, a soluble polypeptide of a molecular mass of 140 kDa. This fragment originates from the amino-terminal part of the molecule (4). Further digestion of glycocalicin with trypsin yields a highly glycosylated polypeptide fragment termed "macroglycopeptide" with a molecular mass of 118 kDa (5) and a smaller fragment with a molecular mass of 45 kDa. The 45-kDa fragment includes the amino terminus of GPIbα and contains the binding sites for vWF (6, 7) and thrombin (6, 8).

GPIb contains a large amount of carbohydrate, which is present on both GPIbα and GPIbβ. This carbohydrate is predominantly in the form of O-linked hexasaccharides rich in sialic acid (9–11). The O-linked carbohydrate chains are located almost exclusively in the macroglycopeptide portion of GPIbα. Smaller amounts of N-linked oligosaccharide chains are also present on both chains.

GPIb forms a macromolecular complex within the platelet plasma membrane with GPIX, a glycoprotein of M_r 17,000 (12, 13). GPIb is also associated with actin filaments of the membrane skeleton through an interaction with an actin-binding protein (14, 15). This complex is apparently disrupted by proteolysis of the actin-binding protein during platelet activation (16).

Two lines of evidence support the importance of GPIb in adhesion. First, patients with Bernard–Soulier syndrome suffer from a severe and potentially fatal bleeding diathesis characterized by defective platelet adhesion (17). The Bernard–Soulier syndrome is an autosomal recessive disorder marked by the deficiency of GPIb, GPV, and GPIX (18). Second, platelet adhesion to a vWF-containing collagen matrix under conditions of high shear can be abolished by preincubation of the platelets with a monoclonal antibody raised against GPIb (19).

The glycocalicin moiety of GPIb contains two binding sites for thrombin, including one of high affinity and one of low affinity (8). The function of thrombin binding to GPIb is unclear. Platelets from patients with Bernard–Soulier syndrome show a diminished response to thrombin but can still be activated at high thrombin concentrations (20).

Studies on the molecular biology of platelet proteins have been hampered by difficulties in isolating megakaryocytes in large amounts and by the absence of significant amounts of mRNA in platelets. Martin and Papayannopoulou (21) have recently described an undifferentiated human erythroleukemia cell line, HEL, that expresses platelet proteins, including

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: GP, platelet membrane glycoprotein; GPIbα and GPIbβ, α and β chains of GPIb, respectively; vWF, von Willebrand factor; LRG, leucine-rich α_2-glycoprotein.

¶To whom correspondence should be addressed at: Seattle VAMC (111), 1660 S. Columbian Way, Seattle, WA 98108.

platelet membrane proteins GPIIb/IIIa. HEL cells also express a molecule on their surface that is immunologically similar or identical to platelet GPIbα (22–24), and expression of this GPIb antigen is enhanced following induction of HEL cells with phorbol 12-myristate 13-acetate (23). In the present studies, HEL cells induced with phorbol 12-myristate 13-acetate were chosen as a source of mRNA to construct a λgt11 cDNA expression library. This library was then employed for the isolation of cDNA clones coding for GPIbα.[‖]

MATERIALS AND METHODS

Bacterial alkaline phosphatase, T4 DNA ligase, the Klenow fragment of *Escherichia coli* DNA polymerase I, and nuclease BAL-31 were purchased from Bethesda Research Laboratories. dATP[α-^{35}S] and deoxynucleotide triphosphate/dideoxynucleotide triphosphate sequencing mixes were obtained from Amersham.

Glycocalicin was purified to homogeneity from human platelets by a two-step procedure utilizing affinity chromatography on wheat germ agglutinin-Sepharose and monoclonal antibody (C7E10)-Sepharose columns as described previously (25). Purified glycocalicin was used to immunize rabbits, and the antibodies were purified from the serum by ammonium sulfate precipitation and affinity purification on glycocalicin-Sepharose. The purified antibodies were shown to be monospecific by immunoblotting of whole platelet lysate. The antibodies were radiolabeled with Na^{125}I using IODO-GEN (Pierce) to a specific activity of 1×10^6 cpm/μg (26).

Purified glycocalicin was reduced, carboxymethylated, and digested with trypsin. Peptides were separated by HPLC on an Altex C3 column using a linear gradient system composed of 0.1% trifluoroacetic acid and 0.08% trifluoroacetic acid in 80% acetonitrile. A total of six distinct peptides were purified to homogeneity and sequenced with a Beckman 890C sequencer. A second sample of glycocalicin was digested with *Staphylococcus aureus* V8 protease using the same conditions used for trypsin, and four homogeneous peptides were sequenced.

mRNA was extracted from HEL cells using a modification of the guanidine isothiocyanate method (27) after induction of the cells for 48 hr with 160 nM phorbol 12-myristate 13-acetate. Poly(A)-RNA was isolated by chromatography on oligo(dT) cellulose (28), and 5 μg of RNA was used for the preparation of a λgt11 cDNA library as previously described (29).

Recombinant phage were initially screened with the ^{125}I-labeled affinity-purified antibody to glycocalicin by the method of Young and Davis (30). Positive phage were identified and plaque-purified. A 1.1-kilobase cDNA coding for GPIb was then radiolabeled with ^{32}P by nick-translation and was used to rescreen the cDNA library.

Phage DNA was prepared by the liquid/lysis method and purified by cesium chloride banding (31, 32). The cDNA inserts were isolated by digestion with *Eco*RI and subcloned into pUC18. The inserts were then digested with appropriate restriction enzymes or with exonuclease BAL-31 (33) and were subcloned into phage cloning vectors M13mp18 and M13mp19 for DNA sequencing. Sequence analysis was performed by the dideoxy chain termination method (34) using deoxyadenosine 5′-[α-[^{35}S]thio]triphosphate. Sequences were stored and analyzed using the GENEPRO computer program (Riverside Scientific, Seattle).

[‖]The sequence of one of these clones, λGPIb2.4, is being deposited in the EMBL/GenBank data base (Bolt, Beranek, and Newman Laboratories, Cambridge, MA, and Eur. Mol. Biol. Lab., Heidelberg) (accession no. J02940).

RESULTS AND DISCUSSION

Four million recombinant phage from the HEL cell λgt11 expression library were screened with the ^{125}I-labeled affinity-purified antibody to glycocalicin. One of the positive clones (λGPIb1.1) was plaque-purified and its cDNA insert was sequenced by the dideoxy chain termination method. DNA sequence analysis of this clone revealed that it coded for a polypeptide with an amino acid sequence that was identical to that obtained from the peptides of glycocalicin. The cDNA insert was then nick-translated and used as a hybridization probe to rescreen the HEL cell cDNA library. Ten additional positive clones were identified and six were plaque-purified. Three clones contained cDNA inserts that were approximately 2.5 kilobases in length. One of these clones, λGPIb2.4, was subjected to DNA sequence analysis. The restriction map and cloning strategy for the two clones (λGPIb1.1 and λGPIb2.4) are shown in Fig. 1. Over 90% of the sequence was determined for both strands. The nucleotide sequence and predicted amino acid sequence for the cDNA insert coding for human GPIbα are shown in Fig. 2. The cDNA was 2420 nucleotides in length and included a short 5′ untranslated segment of 42 nucleotides followed by a region coding for a signal peptide of 16 amino acids and a mature protein of 610 amino acids. A TGA stop codon (nucleotides 1921–1923) was followed by a 3′ noncoding region of 497 nucleotides. The 3′ noncoding sequence included a polyadenylylation signal of AATAAA that was located 19 nucleotides upstream from the poly(A) tail.

The initiator methionine was followed by a putative signal peptide of 16 hydrophobic residues. This signal peptide is unusual in that it has a proline at position −1. This is very rare and is thought to be incompatible with correct signal processing (35). The amino acid sequence of the mature protein predicted from the cDNA agrees entirely with that obtained by Edman degradation of the peptides prepared from human platelet glycocalicin (underlined in Fig. 2). The extensive sequence identity provides strong evidence that GPIbα derived from HEL cells is the same as that from platelets. The amino acid sequence predicted by the cDNA is also consistent with that published by Handa *et al.* (7), who reported an amino-terminal sequence for GPIbα (His-Pro-Ile; H P I) and two other sequences for fragments obtained by digestion with *S. aureus* V8 protease. It also agrees completely with that determined by Edman degradation of platelet glycocalicin by Titani *et al.* (36). The nucleotide sequence of λGPIb1.1 and

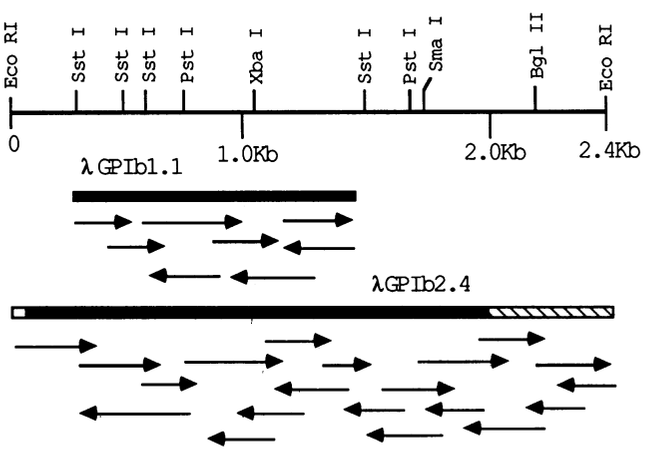

FIG. 1. Restriction map and cloning strategy for two cDNA inserts coding for human GPIbα. The length and direction of sequencing are indicated by the arrows under the respective clones. The 5′ untranslated sequence is indicated by an open bar, the coding sequence by a solid bar, and the 3′ untranslated sequence by a hatched bar.

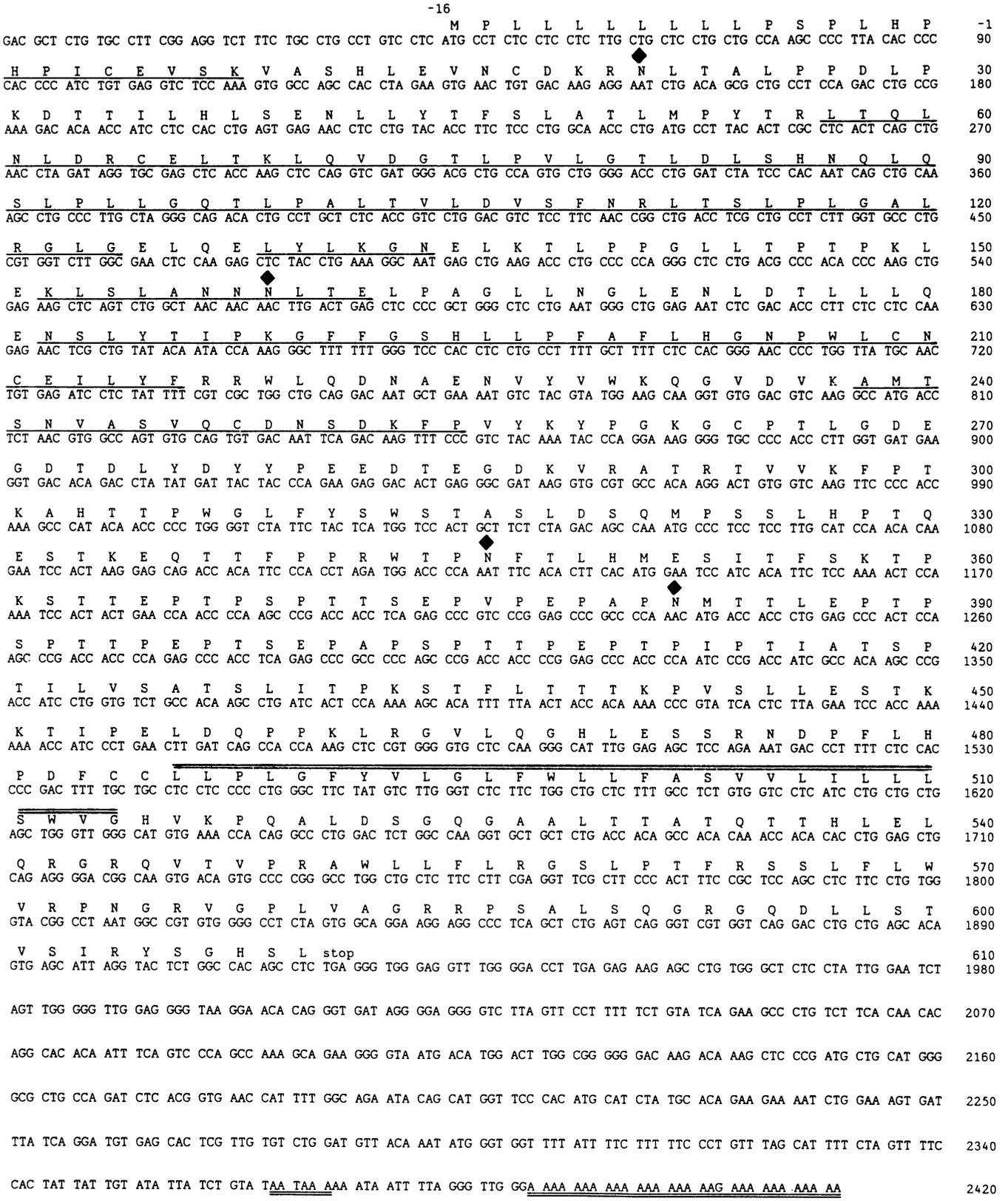

FIG. 2. Nucleotide sequence and predicted amino acid sequence for the cDNA insert coding for human GPIbα. The amino terminus of the mature protein starts with +1, whereas the negative numbers (−16 through −1) represent the signal sequence. Amino acid sequences obtained by Edman degradation are shown with a single underline, and the polyadenylylation or processing signal and poly(A) tail are shown with a double underline. The potential transmembrane region is identified with a double overline. Potential N-linked glycosylation sites are shown by solid diamonds.

λGPIb2.4 differed for codon 396—i.e., GAG (glutamic acid; E) in the former (shown in Fig. 2) and GGC (glycine; G) in the latter. This is probably the result of a cloning artifact but may also represent an allelic polymorphism. The size of the cDNA coding for GPIbα was 2420 nucleotides, which is in good agreement with the size of the mRNA estimated at 2.5 kilobases by blot hybridization of total HEL cell mRNA (data not shown).

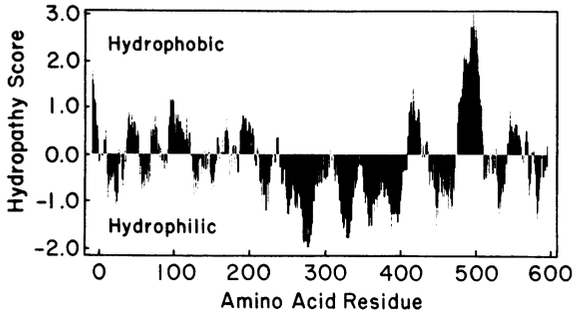

FIG. 3. Hydropathy plot of the amino acid sequence of human GPIbα employing the method of Kyte and Doolittle (37) using a window of 15 residues. The two major hydrophobic stretches (−1 to −15 and 486 to 514) represent the signal peptide and the putative transmembrane domain, respectively.

Four potential asparagine-linked glycosylation sites (N X S/T) are present in the molecule and are located at residues 21, 159, 346, and 382. A reduction in molecular mass of 12 kDa occurs for GPIbα when HEL cells are grown in the presence of tunicamycin (24). This suggests that carbohydrate is probably attached to all of the four potential binding sites.

On the basis of the predicted amino acid sequence, the amino acid composition for the mature protein is calculated to be: $Ala_{26}Cys_{9}Asp_{24}Glu_{31}Phe_{22}Gly_{35}His_{14}Ile_{12}Lys_{26}Leu_{100}$-$Met_{5}Asn_{23}Pro_{63}Gln_{22}Arg_{23}Ser_{49}Thr_{70}Val_{32}Trp_{10}Tyr_{14}$. This yields a molecular mass of 67,192 for the protein freed of carbohydrate. This composition is in good agreement with that obtained for GPIbα from a 24-hr acid hydrolysate.

Hydropathy analysis (37) of the amino acid sequence of GPIbα revealed several hydrophobic domains in the molecule (Fig. 3). These include a signal peptide at the amino terminus and a putative membrane-spanning segment, which is approximately 29 amino acids in length (double overline in Fig. 2). The potential membrane-spanning region is flanked by charged amino acids, which may serve to anchor the protein in the membrane. Two major hydrophilic regions are present in GPIbα. The first begins at about residue 250 and extends approximately 60 residues towards the carboxyl terminus. This region is rich in highly charged amino acids, including aspartic acid, lysine, arginine, and glutamic acid. Within this stretch of amino acids, there is an abrupt transition at residue 288 from positively charged to negatively charged amino acids. The second major hydrophilic stretch (residues 320–420) is rich in serine and threonine and probably contains the majority of the O-linked carbohydrate. This region also contains five repeats of nine amino acids with a consensus sequence of T T – E P T P – P (Fig. 4 *Upper*). A computer-assisted search of the Protein Sequence Database** revealed that many proteins have sequences similar to this region of GPIbα. These proteins include a number of viral envelope and nucleocapsid glycoproteins as well as several eukaryotic cell surface proteins. These similarities may indicate a conserved signal for O-linked glycosylation.

GPIbα contains nine cysteine residues, all of which are present in the apparent extracytoplasmic domain. Two cysteine residues are located immediately preceding the putative membrane-spanning segment. One (or both) of these cysteine residues may form a disulfide bond(s) with the β chain of GPIb. It is unlikely that either of these cysteine residues participates in intrachain disulfide bonds, as this would prevent the release of glycocalicin from GPIbα by limited proteolysis without reduction. The remaining seven cysteine residues are located in the glycocalicin portion of the molecule; one possibility is that they form three intrachain disulfide bonds, and one residue remains as a free cysteine.

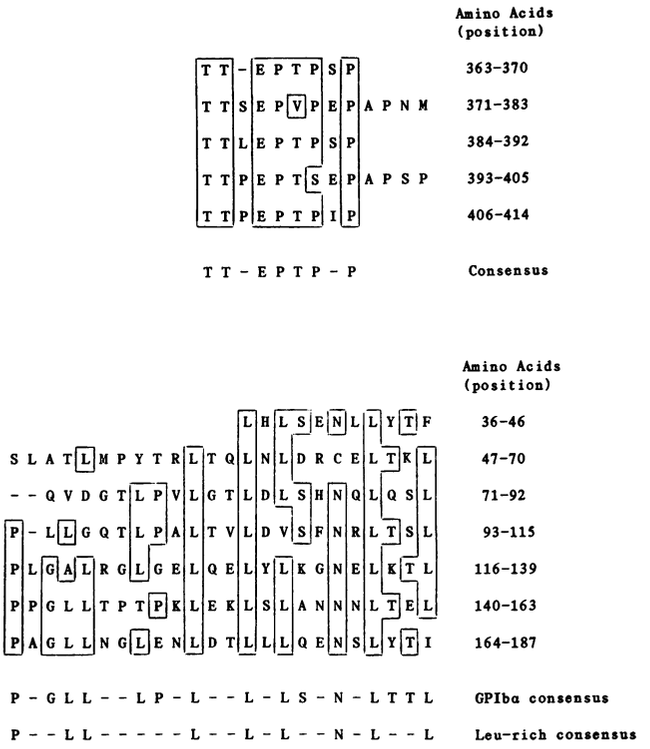

FIG. 4. Tandem repeats in the amino acid sequence of human GPIbα. Consensus sequences derived from GPIbα and LRG (38) are also shown. Residues present three or more times in a given position are boxed. (*Upper*) Amino acid repeats between residues 363 and 414. (*Lower*) Amino acid repeats between residues 36 and 187.

The amino-terminal portion of GPIbα contains the vWF binding site within a 45-kDa proteolytic fragment (6, 7). If the two N-linked carbohydrate side chains were approximately 3 kDa in size, a proteolytic cleavage at either Lys-334 or Arg-342 would generate tryptic fragments of 43 kDa and 44 kDa, respectively. This amino-terminal fragment also contains seven tandem repeats of 24 amino acids. These repeats are homologous to a consensus sequence of P – – L L – – – – – L – – L – L – – N – L – – L, which is derived from 13 tandem repeats in leucine-rich α_2-glycoprotein (LRG) of plasma (38). An alignment of these leucine-rich repeats present in GPIb is shown in Fig. 4 *Lower*. Thus far, no physiological function for LRG has been reported.

The nucleotide sequence for GPIbα predicts an intracytoplasmic domain of approximately 100 residues. This segment is thought to be associated with the membrane skeleton through an interaction with actin-binding protein (14). Although several potential phosphorylation sites (serine, threonine, and tyrosine) are present in this segment, no evidence exists that they become phosphorylated.

The predicted sequence of GPIbα is not homologous with the adhesive receptors belonging to the "integrin" or "cytoadhesin" gene family. This family includes platelet GPIIb/IIIa, vitronectin, and fibronectin receptors, the chicken fibroblast fibronectin receptor integrin (CSAT antigen), and a group of leukocyte adhesion receptors (LFA-1, Mac-1, p150, and 90) (39). A protein immunologically related to platelet GPIb is present on endothelial cells and displays ristocetin-dependent binding of vWF (40, 41). The function of this protein, however, is not known.

The present data indicate that GPIbα and GPIbβ are coded by different mRNAs generated from two different genes. The two chains do not arise as a result of posttranslational processing of a precursor protein. It is also possible that the

**Protein Identification Resource (1986) Protein Sequence Database (Natl. Biomed. Res. Found., Washington, DC), Release 10.0.

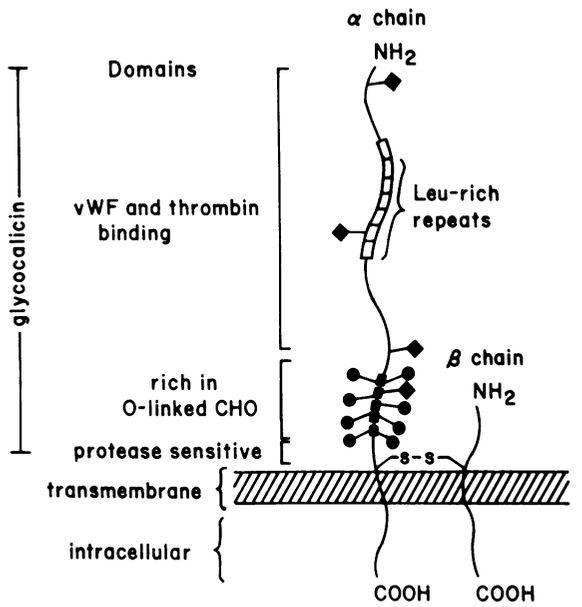

FIG. 5. Tentative representation for the α and β chains of human GPIb based on current information. The potential O-linked and N-linked carbohydrate chains are shown as solid circles and solid diamonds, respectively. The number of O-linked chains is underrepresented here. The Leu-rich tandem repeats are shown by open bars, and the repeats of nine amino acids are shown by solid bars [modified from Wicki and Clemetson (6)].

developmental regulation of GPIbα and GPIbβ is independent, since GPIbα can be expressed in the absence of GPIbβ in HEL cells (24). The function of GPIbβ and its role in GPIb remains to be determined.

In this study, the entire amino acid sequence for human platelet GPIbα has been established. These data strongly suggest that GPIbα is an integral membrane glycoprotein with an extracytoplasmic domain of approximately 485 amino acids, a transmembrane segment of approximately 29 amino acids, and an intracellular domain of approximately 100 amino acids (Fig. 5). The extracytoplasmic portion of GPIbα also contains the seven repeating sequences that are homologous to the repeating sequence present in LRG.

We thank Lee Hendrickson, Brad McMullen, Mark Hickey, Stuart Williams, and Connie Ward for excellent technical assistance and Dr. Diane Nugent for the generous gift of monoclonal antibody C7E10. This investigation was supported in part by a National Institutes of Health research grant (HL 16919), a grant from the Robert Wood Johnson Foundation (no. 10787), a Grant-in-Aid from the American Heart Association, and a Merit Review Grant from the Veterans Administration. J.A.L. is a Fellow of the Robert Wood Johnson Foundation and D.W.C. and G.J.R. are Established Investigators of the American Heart Association.

1. Phillips, D. R. & Agin, P. P. (1977) *J. Biol. Chem.* **252,** 2121–2126.
2. Clemetson, K. J., Naim, H. Y. & Luscher, E. F (1981) *Proc. Natl. Acad. Sci. USA* **78,** 2712–2716.
3. Phillips, D. R. & Jakabova, M. (1977) *J. Biol. Chem.* **252,** 5602–5605.
4. Okumura, T. & Jamieson, G. A. (1976) *J. Biol. Chem.* **251,** 5944–5949.
5. Okumura, T., Lombart, C. & Jamieson, G. A. (1976) *J. Biol. Chem.* **251,** 5950–5955.
6. Wicki, A. N. & Clemetson, K. J. (1985) *Eur. J. Biochem.* **153,** 1–11.
7. Handa, M., Titani, K., Holland, L. Z., Roberts, J. R. & Ruggeri, Z. M. (1986) *J. Biol. Chem.* **261,** 12579–12585.
8. Okumura, T., Hasitz, M. & Jamieson, G. A. (1978) *J. Biol. Chem.* **253,** 3435–3443.
9. Judson, P. A., Anstee, D. J. & Clamp, J. R. (1982) *Biochem. J.* **205,** 81–90.
10. Tsuji, T., Tsunehisa, S., Watanabe, Y., Yamamoto, K., Tohyama, H. & Osawa, T. (1983) *J. Biol. Chem.* **258,** 6335–6339.
11. Korrel, S. A. M., Clemetson, K. J., Van Halbeek, H., Kamerling, J. P., Sixma, J. J. & Vliegenthart, J. F. G. (1984) *Eur. J. Biochem.* **140,** 571–576.
12. Berndt, M. C., Gregory, C., Kabral, A., Zola, H., Fournaier, D. & Castaldi, P. A. (1985) *Eur. J. Biochem.* **151,** 637–649.
13. Wicki, A. N., Schnippering, W. & Clemetson, K. J. (1985) *Thromb. Haemostasis* **54,** 6.
14. Fox, J. E. B. (1985) *J. Biol. Chem.* **260,** 11970–11977.
15. Okita, J. R., Pidard, D., Newman, P. J., Montgomery, R. R. & Kunicki, T. J. (1985) *J. Cell Biol.* **100,** 317–321.
16. Fox, J. E. B., Reynolds, C. C. & Phillips, D. R. (1983) *J. Biol. Chem.* **258,** 9973–9981.
17. Bernard, J. & Soulier, J. P. (1948) *Sem. Hop. Paris* **24,** 3217–3223.
18. Clemetson, K. J., McGregor, J. L., Dechavanne, J. E. & Luscher, E. F. (1982) *J. Clin. Invest.* **70,** 304–311.
19. Sakariassen, K. S., Nievelstien, P. F., Coller, B. S. & Sixma, J. J. (1986) *Br. J. Haematol.* **63,** 681–691.
20. Jamieson, G. A. & Okumura, T. (1978) *J. Clin. Invest.* **61,** 861–864.
21. Martin, P. & Papayannopoulou, T. (1982) *Science* **216,** 1233–1235.
22. Papayannopoulou, T., Yokochi, T., Nakamoto, B. & Martin, P. (1983) in *Globin Gene Expression and Hematopoietic Differentiation*, eds. Stamatoyannopoulos, G. & Nienhuis, A. W. (Liss, New York), pp. 277–292.
23. Tabilio, A., Rosa, J. P., Testa, U., Breton-Gorius, J. & Vainchenker, W. (1984) *EMBO J.* **3,** 453–459.
24. Kieffer, N., Debili, N., Wicki, A., Titeux, M., Henrri, A., Mishal, Z., Breton-Gorius, J., Vainchenker, W. & Clemetson, K. J. (1986) *J. Biol. Chem.* **261,** 15854–15862.
25. Canfield, V. A., Ozols, J. & Roth, G. J. (1985) *Blood* **66,** Suppl. 1, 301a (abstr.).
26. Salacinski, P. R. P., McLean, C., Sykes, J. E. C., Clement-Jones, V. V. & Lowry, P. J. (1981) *Anal. Biochem.* **117,** 136–146.
27. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
28. Aviv, H. & Leder, P. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 1408–1412.
29. Hagen, F. S., Gray, C. L., O'Hara, P., Grant, F. J., Saari, G. C., Woodbury, R. G., Hart, C. E., Insley, M., Kisiel, W., Kurachi, K. & Davie, E. W. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2412–2416.
30. Young, R. A. & Davis, R. W. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1194–1199.
31. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY), pp. 76–85.
32. Leytus, S. P., Kurachi, K., Sakariassen, K. S. & Davie, E. W. (1986) *Biochemistry* **25,** 4855–4863.
33. Poncz, M., Solowiejczyk, D., Ballantine, M., Schwartz, E. & Surrey, S. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4298–4302.
34. Sanger, F., Nicklen, S. & Coulson, A. R. (1977) *Proc. Natl. Acad. Sci. USA* **74,** 5463–5467.
35. Von Heijne, G. (1983) *Eur. J. Biochem.* **133,** 17–21.
36. Titani, K., Takio, K., Handa, M. & Ruggeri, Z. M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 5610–5614.
37. Kyte, J. & Doolittle, R. F. (1982) *J. Mol. Biol.* **157,** 105–132.
38. Takahashi, N., Takahashi, Y. & Putnam, F. W. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 1906–1910.
39. Hynes, R. O. (1987) *Cell* **48,** 549–555.
40. Spandio, J. D., Thiagarajan, P. & Shapiro, S. S. (1986) *Blood* **68,** Suppl. 1, 357a (abstr.).
41. Asch, A. S., Fujimoto, M., Adelman, B. & Nachman, R. (1986) *Circulation* **74,** Suppl. 2, 232a (abstr.).