Structural Characterization and Chromosomal Location of the Gene Encoding Human Platelet Glycoprotein Ibb*

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Human platelet glycoprotein Ibb (GPIbb) (M, 22,000) is part of the GPIb-V-IX system that constitutes the receptor for von Willebrand factor and mediates platelet adhesion in the arterial circulation. The four members of the receptor (GPs Iba, Ibb, V, and IX) share structural and functional features. Individually, GPIbb contributes to surface expression of the receptor and participates in transmembrane signaling through phosphorylation of its intracellular domain. To define the structure of the GPIbb gene, a cosm id clone from a human genomic library was analyzed. The transcriptional start site was located by both primer extension and the "anchored" polymerase chain reaction. Similar to the genes for Iba, V, and IX, the Ibb gene is compact with a single 274-base intron inserted into the 5' end of the open reading frame. The 5'-flanking region of the gene contains both GATA and ets sites that are also found in the 5' promoter regions of other described megakaryocyte/platelet genes. The GPIbb gene was localized to chromosome 22q11.2 by fluorescence in situ hybridization. The GPIbb gene has a simple structure, similar to that of other described megakaryocyte/platelet genes, including those of the GPIb-V-IX system.

Human platelet glycoprotein Ibb (GPIbb) is a subunit of the receptor for von Willebrand factor (1, 2). The initial step in the adhesion of platelets to injured arterial walls depends on the interaction of the receptor (GPIb-V-IX) with its ligand (von Willebrand factor (3). The receptor consists of four physically associated proteins. GPIbα (M, 143,000) is linked through a disulfide bond(s) to GPIbb (M, 22,000) producing the GPIb heterodimer, which, in turn, is noncovalently bound to the two other members, GPV (M, 83,000) and GPIIX (M, 20,000) (1, 4, 6). The four elements share a common structural motif, the leucine-rich glycoprotein segment (7); surface expression of all four chains is altered in Bernard-Soulier syndrome, a congenital disorder of the receptor that occurs in both classic and variant forms (5, 8-10). The receptor has a discrete binding site for the von Willebrand factor ligand, located within the GPIbα chain (11).

Individually, the GPIbb chain contributes to the expression of a functional receptor; transfection studies demonstrate that the GPIbb cDNA, along with that for GPIIX, is required for efficient expression of surface GPIbb (12). In addition, GPIbb may participate in transmembrane signaling through phosphorylation of its intracellular domain (13).

The four receptor proteins are encoded by distinct transcripts as defined by cDNA cloning studies (14-17), and the GPIbα and IX genes are located on different chromosomes (18, 19). However, the GPIbα, V, and IX genes share consensus cis-acting promoter sequences (GATA and ets sites) along with structural features such as compact size, relatively few introns, and continuous (intron-less) open reading frames (18-21). In the current study, we set out to characterize the structure and chromosomal location of the GPIbb gene.

MATERIALS AND METHODS

Southern Blotting and Cosmid Cloning—Following restriction digestion, DNA fragments were electrophoresed on 0.8% agarose, transferred to nitrocellulose, and hybridized to a probe that was generated by PCR amplification of the 3'-untranslated region of the GPIbb cDNA (nucleotides 628-950) (15) and labeled by random priming. The same probe was used to screen 5 x 107 colonies of a human genomic cosm id library (Stratagene, fetal brain) (20). Positive clones were colony-purified, and one of these was further analyzed. Restriction fragments were subcloned into Bluescript SK+ (Stratagene), and DNA sequencing was performed using Sequenase version 2.0 (U.S. Bioc hemical Corp.). Sequencing analysis was performed using PCCGENE (IntelliGenetics).

Primer Extension—RNA was extracted from the human erythroblastic cell line, K562, or human platelets using acid guanidinum isothiocyanate (22, 23). Poly(A)+ RNA was selected with oligo(dT),,.,, cellulose. The primers PEB1 (complementary to bases 47-71) (15) and PEI2 (complementary to bases 115-138) were labeled with T4 polynucleotide kinase (Boehringer Mannheim) to a specific activity of 2.6 x 106 dpm/μg (2.0 x 105 dpm/μl). Each oligonucleotide (105 dpm, 0.05 pmol) was annealed to 0.5 μg poly(A)+ RNA or 10 μg total K562 RNA (16 h, 32°C, 150 mM KCl, 1 mM EDTA, 10 mM Tris, pH 7.5), and extended using reverse transcriptase (Life Technologies, Inc.; Super-Script) under the conditions recommended by the manufacturer. After RNAse digestion, products were phenol-extracted, ethanol-precipitated, separated on an 8% polyacrylamide sequencing gel, and autoradiographed. A DNA sequencing reaction of M13mp18 was used as a size standard.

Amplification of mRNA 5' Ends—The 5' ends of GPIbb mRNA were analyzed by the protocols and reagents of the Clontech AmpliFINDER kit. Using platelet poly(A)+ RNA (2 μg) and the PEI2 or PEI3 (bases 172-196) (15) primers, single-stranded cDNA was synthesized and then purified after hydrolysis of the RNA template. A short oligonucleotide (90mer) was ligated to the cDNA using T4 RNA ligase. The 5' ends of the GPIbb cDNAs were amplified by PCR using PEI1 as the antisense primer, and either a complementary anchor oligonucleotide or an oligonucleotide corresponding to bases 1-18 (see Ref. 15) as the sense primer. Amplified products were separated by electrophoresis on a 4% NuSieve agarose (3:1 gel), isolated on NA-45 membranes (Schleicher & Schuell), cloned into the pCR1 vector (Invitrogen), and sequenced.
Southern blot analysis of normal human genomic DNA (10 μg) was digested with restriction endonucleases (E, EcoRI; B, BamHI; H, HindIII; P, PstI; X, XbaI; S, SacI). Fragments were separated by electrophoresis, transferred to nitrocellulose, hydridized to a labeled GPIbp cDNA probe, and detected by autoradiography. Numbers at left (kb) indicate the migration of the hybridization was carried out as previously described by Edelhoff et al. (24). Hybridization signals were detected with a commercial system derived from the 3'-untranslated region (UTR) of the platelet GPIbp gene at the positions indicated in Fig. 2. The transcriptional start site for GPIbp was mapped by two primer extension experiments were performed on procedures. Primer extension experiments were performed on primer extension, cDNA synthesis, and PCR (PEB1, PEB2, PEB3) are confirmed by using a primer antisense primer and sense primers specific for addition of the oligonucleotide and amplification with its primer adds 48 bases to the end of the mRNA was confirmed within a glycine codon for the fourth amino acid residue of the predicted signal peptide (15). Further sequencing (not shown) indicates that the second exon includes the entire 3'-UTR; thus, like the genes for the other members of this family, the GPIbp gene includes the coding sequence for the mature protein and the 3'-UTR in a single exon.

The transcriptional start site for GPIbp was mapped by two procedures. Primer extension experiments were performed on platelet or K562 RNA using two primers that hybridize to the GPIbp gene at the positions indicated in Fig. 2. As shown in Fig. 3A, these primers give rise to specific bands in platelet but not in K562 RNA. K562 cells do not contain GPIbp transcripts (based on data similar to that showing the absence of GPV transcripts in HEL cell sublines) (17), despite their ability to express the genes for GPIIX and GPIIIb (20, 25). The sizes of the platelet-related products (major 76 bases, minor 72/78 for PEB1; and major 140, minor 137/142 for PEB2) are consistent with the size predicted by the previously reported cDNA sequence (15). The other bands present in both the K562 and platelet lanes are present also in a yeast tRNA control and are thought to be nonspecific products. The major transcriptional start site defined by the primer extension experiments lies 5 bases upstream from the 5' end of the reported cDNA sequence (15). The identification of the 5' end of the mRNA was confirmed by using a 5' "rapid amplification of cDNA ends" protocol (2B). cDNA was synthesized using the primers PEB2 or PEB3, ligated to a synthetic oligonucleotide, and amplified with PEB1 antisense primer and sense primers specific for either the ligated oligonucleotide (lanes 2 and 4) or the identified GPIbp 5'-UTR (lanes 1 and 3). Addition of the oligonucleotide and amplification with its primer adds 48 bases to the end of the cDNA, consistent with the difference in sizes of the respective bands in Fig. 3B. Sequence analysis of clones isolated from the amplified products confirmed that transcription of the GPIbp gene begins in the same region and at the same sites determined by primer extension. The degree of heterogeneity in the transcriptional start site, shown in Fig. 3, A and B, is characteristic of many promoters that lack an identifiable TATAA box (26). Inspection of the 5'-flanking sequence reveals the presence of several potential binding sites for transcription factors. A rec-
Fig. 3. The start site for transcription of the GPIbβ gene. A, primer extension analysis. Human erythroleukemia cell K562 (10 µg total, lane 1) and platelet (0.5 µg poly(A), lane 2) RNA was annealed to radiolabeled oligonucleotide primers (PEB1, right; PEB2, left), extended with reverse transcriptase, digested with RNase A, analyzed by electrophoresis on a denaturing polyacrylamide gel, and autoradiographed. Labeled DNA products were sized by comparison to a DNA sequencing ladder of M13 mp18.

Fig. 4. Diagram of the GPIbβ gene. The upper line represents approximately 8 kb of human genomic DNA with restriction sites denoted as described in Fig. 1. The region of the GPIbβ gene is enlarged in the lower line drawing, with exons 1 and 2 denoted by boxes, 36 and 918 bases in size. The intervening sequence (IVS-1, intron) of 274 bases is noted, and the divided open reading frame (10 and 608 bases) is indicated by the hatched enclosures.

Fig. 5. Assignment of the GPIbβ gene to human chromosome 22 band q11.2 as determined by fluorescence in situ hybridization. The biotinylated GPIbβ cosmids was hybridized to fixed metaphase chromosomal spreads and detected with goat anti-biotin/fluoresceinated rabbit anti-goat antibody. Chromosomes stained with Hoechst 33258-actinomycin D were counterstained with propidium iodide. Top, fluorescence micrographs of one field. a, hybridization; b, banding. The probe hybridized to chromosome 22q11.2 as indicated by arrows and identified by banding. Bottom, the distribution of 42 hybridization signals is shown in a diagram of human chromosome 22.

29–32). A CG-rich sequence recognized by SP-1 is located at −54 in the GPIbβ gene (Fig. 2), and a similar SP-1 site is found in the 5′ promoter region of the GPIX gene (20, 33). Like other platelet/megakaryocyte genes, the promoter region of the GPIbβ gene lacks both CCAAT and TATAA boxes. However, unlike the oligodendrocyte-myelin protein and GPIba related transcript was detected in endothelium, brain, heart, and placenta. Endothelial, brain, and heart cells may utilize different promoters and start sites that are located upstream from the platelet GPIbβ gene, and these cells may process the resultant transcript in a manner that...
differs from that of the megakaryocyte. The protein produced from this alternative transcript is approximately twice as large as platelet GPIββ (35).

The GPIββ gene was mapped on human metaphase chromosomes by fluorescence in situ hybridization using the described human cosmid clone as probe. Of 107 cells examined, 42 (39%) showed signals on both chromatids of one or both chromosomes 22 at band q11.2. In addition, 27 (25%) showed signals on one chromatid at the same site (Fig. 5). No significant hybridization to other chromosomes was observed, indicating that the human platelet GPIββ gene is located on chromosome 22q11.2. The GPIββ gene does not form a cluster with the GPIba and IX genes, because these are located on chromosomes 17 and 3, respectively (18, 19).

In summary, the human platelet GPIββ gene is compact in size with a simple structure that resembles that of other leucine-rich glycoprotein genes such as those of the Ib-V-IX system with a single intron inserted into the 5' end of its open reading frame. The gene shares putative cis-acting promoter elements (GATA/ets/SP-1) with related "megakaryocyte/platelet" genes and is located on chromosome 22q11.2. The GPIββ gene does not form a cluster with the GPIba and IX genes, because these are located on chromosomes 17 and 3, respectively (18, 19).

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REFERENCES