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Comparison of cDNA-Derived Protein Sequences of the Human Fibronectin and Vitronectin Receptor α-Subunits and Platelet Glycoprotein IIb†

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Received June 10, 1987; Revised Manuscript Received August 4, 1987

ABSTRACT: The fibronectin receptor (FnR), the vitronectin receptor (VnR), and the platelet membrane glycoprotein (GP) IIb-IIIa complex are members of a family of cell adhesion receptors, which consist of noncovalently associated α- and β-subunits. The present study was designed to compare the cDNA-derived protein sequences of the α-subunits of human FnR, VnR, and platelet GP IIb. cDNA clones for the α-subunit of the FnR (FnR_α) were obtained from a human umbilical vein endothelial (HUVE) cell library by using an oligonucleotide probe designed from a peptide sequence of platelet GP IIb. cDNA clones for platelet GP IIb were isolated from a cDNA expression library of human erythroleukemia cells by using antibodies. cDNA clones of the VnR α-subunit (VnR_α) were obtained from the HUVE cell library by using an oligonucleotide probe from the partial cDNA sequence for the VnR_α. Translation of these sequences showed that the FNR_α, the VnR_α, and GP IIb are composed of disulfide-linked large (858-871 amino acids) and small (137-158 amino acids) chains that are posttranslationally processed from a single mRNA. A single hydrophobic segment located near the carboxyl terminus of each small chain appears to be a transmembrane domain. The large chains appear to be entirely extracellular, and each contains four repeated putative Ca^{2+}-binding domains of about 30 amino acids that have sequence similarities to other Ca^{2+}-binding proteins. The identity among the protein sequences of the three receptor α-subunits ranges from 36.1% to 44.5%, with the Ca^{2+}-binding domains having the greatest homology. These proteins apparently evolved by a process of gene duplication.

Cell adhesion and platelet aggregation are mediated by the binding of adhesive ligands, such as fibronectin and fibrinogen, to a family of cell membrane receptors (Ruoslahti & Pierschbacher, 1986; Hynes, 1987). These adhesion receptors consist of heterodimer complexes of noncovalently linked α-subunits (M_r 140 000-200 000) and β-subunits (M_r 95 000-120 000). Although there are at least 10 receptors in this family, there are only 3 known β-subunits, each of which defines a subfamily of receptors (Hynes, 1987). One subfamily consists of the platelet glycoprotein (GP)[1] IIb-IIIa complex, which is required for platelet aggregation, and the widely

† This research was supported by National Institutes of Health Grants HL 28947 and HL 32254 (to D.R.P.).
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[1] Abbreviations: GP, glycoprotein; VnR, vitronectin receptor; FnR, fibronectin receptor; FnR_α, fibronectin receptor α-subunit; VLA, very late antigens; SDS, sodium dodecyl sulfate; VnR_α, vitronectin receptor α-subunit; HUVE cell, human umbilical vein endothelial cell; HEL cell, human erythroleukemia cell; SSC, saline-sodium citrate; kDa, kilodalton(s); Tris·HCl, tris(hydroxymethyl)aminomethane hydrochloride.

0006-2960/87/0426-8158$01.50/0

distributed vitronectin receptor (VnR); they share GP IIIa as their β-subunit (Ginsberg et al., 1987). A second subfamily includes the widely distributed human fibronectin receptor (FnR) and the very late antigens (VLA) (first described on stimulated T-lymphocytes), which share a 120-kDa β-subunit (Hemler et al., 1987; Takada et al., 1987a). The five α-subunits in the mammalian FnR/VLA receptor subfamily are distinct proteins, with the FnR α-subunit (FnR_α) having a molecular weight of 160000 on nonreduced sodium dodecyl sulfate (SDS)–polyacrylamide gels. In chickens, there appear to be two separate FnR α-subunits, which have different molecular weights (band 1, M_r 160000; band 2, M_r 140000) and are noncovalently complexed to a β-subunit of M_r 120000 (band 3) (Buck et al., 1985, 1986). A third adhesion receptor subfamily consists of the leukocyte Mac-1, LFA-1, and p150/95 antigens (Springer & Anderson, 1985), which share a 95-kDa β-subunit (Kishimoto et al., 1987). The receptors in this third subfamily mediate the interactions of leukocytes with endothelial cells and with the C3bi fragment of complement (Micklem & Sim, 1985; Springer & Anderson, 1985; Kishimoto et al., 1987). The cDNA and deduced protein sequences for each of these adhesion receptor β-subunits have been determined (Tamkun et al., 1986; Fitzgerald et al., 1987; Kishimoto et al., 1987). These proteins share a number of structural features, including conservation of all 56 cysteines. The β-subunit protein sequences are homologous (38.5–45.4% of the amino acids are identical), indicating that they probably evolved by a process of gene duplication.[2]

The overall protein structure and sequence homology among the α-subunits in the adhesion receptor family are less well understood. These proteins are diverse in size, and some consist of two-chain, disulfide-linked polypeptides (e.g., GP IIb) (Argraves et al., 1986; Suzuki et al., 1986; Hynes, 1987; Poncz et al., 1987). Partial amino-terminal sequences of several α-subunits (GP IIb, VnR_α, VLA, LFA-1, and Mac-1) are ~40% identical (Charo et al., 1986; Suzuki et al., 1986; Takada et al., 1987b). Partial cDNA sequences for the human FnR_α (Argraves et al., 1986) and VnR_α have been reported, and the carboxyl-terminal cDNA-deduced protein sequences are ~40% identical.

This report describes the isolation of full-length human endothelial cell cDNA clones for the FnR_α and of cDNA clones and the sequence corresponding to the amino-terminal region of the VnR_α. The complete deduced protein sequence of the FnR_α aligns with the cDNA-derived protein sequences for platelet GP IIb and the VnR_α. These three α-subunits have a number of common structural features, including the presence of four repeated putative Ca^{2+}-binding domains and a similar positioning of the transmembrane segment. The overall protein sequence homology suggests that they, too, evolved by a process of separate gene duplication.

Experimental Procedures

Isolation of FnR α-Subunit cDNAs. (A) Preparation of GP IIb Peptides and Protein Sequencing. Both the large and small chains of GP IIb were isolated by electroelution from preparative SDS gels of the purified platelet GP IIb–IIIa complex (Fitzgerald et al., 1985a). For cyanogen bromide cleavage, 2.5 mg (30 nmol) of protein was dissolved in 3.3 mL of 70% formic acid and treated with 326 mg of cyanogen bromide for 2 days at ambient temperature. The peptides were first dialyzed against 50% formic acid and then against 0.2 M Tris·HCl/1% SDS, pH 7.87. Ten percent of the dialyzed peptides were dansylated (Stephens, 1975), mixed with the remaining peptides, and fractionated by electrophoresis through a 10–18% SDS–polyacrylamide gel (Laemmli, 1970). Peptide fragments were visualized with UV light and electroeluted from the gel slices. Protein sequences were determined from the amino terminus of the intact GP IIb large and small chains and from three cyanogen bromide fragments of the large chain (approximately 2 nmol of each) using a Beckman 890M sequencer (Beckman Instruments, Fullerton, CA) (Weisgraber et al., 1981).

(B) Preparation of Oligonucleotide Probes. The GP IIb peptide sequences were used to design oligonucleotide probes according to principles of codon use described by Lathe (1985). Probes 42–51 nucleotides in length were synthesized by using an Applied Biosystems (Foster City, CA) 380B synthesizer. The oligonucleotides were purified by electrophoresis through 10% polyacrylamide gels containing 7 M urea and were recovered from gel slices. The probes were labeled on the 5′ end using [γ-^{32}P]ATP (New England Nuclear, Boston, MA) and T4 polynucleotide kinase (Boehringer Mannheim, Indianapolis, IN).

(C) Screening the cDNA Library. A randomly primed human umbilical vein endothelial (HUVE) cell cDNA library was prepared in λgt11 as described (Young & Davis, 1983; Ginsberg et al., 1985). Plaques were screened with the *Escherichia coli* Y1088 strain by hybridization of nitrocellulose filters in 20% formamide, 5 × SSC (SSC is 150 mM NaCl/15 mM sodium citrate), 5 × Denhardt's, 0.1% SDS, and 0.2 mg/mL *E. coli* tRNA at 42 °C (Maniatis et al., 1982). Filters were washed using 2 × SSC at 42–45 °C. Positive plaques were purified and the λ cDNA inserts subcloned into pUC vectors for restriction analysis. The cDNA restriction fragments were subcloned into M13 vectors, and the DNA was sequenced by the dideoxy method of Sanger et al. (1977) using dATP-5′-[α-^{35}S]thiophosphate (New England Nuclear). Sequence data analysis was performed by using the BIONET system of Intelligenetics, Inc. (Palo Alto, CA).

Isolation of GP IIb cDNAs. cDNA clones specific for GP IIb were isolated from a human erythroleukemia (HEL) cell cDNA λgt11 expression library as described (Poncz et al., 1987).

Isolation of VnR_α cDNAs. The published partial cDNA sequence of VnR_α was used to obtain cDNA clones for the VnR_α. A 42-mer oligonucleotide probe corresponding to nucleotides 78–119 of this sequence (Suzuki et al., 1986) was synthesized, purified, ^{32}P-labeled on the 5′ end, and used to screen the randomly primed HUVE cell cDNA library as described above, except that the filters were washed with 2 × SSC at 50–55 °C. Only the 5′ 1323 nucleotides of a VnR_α cDNA clone were sequenced, which included the first *Hin*dIII site in the published sequence.

Results

The FnR_α cDNA clones were isolated from a HUVE cell cDNA library by using an oligonucleotide probe designed from the platelet GP IIb peptide sequence. Figure 1A shows the sequence of this probe, which matched the cDNA sequence in 40 of 51 positions, of which the first 18 nucleotides were correctly assigned. A translation of the cDNA sequence matched the sequence of the platelet GP IIb peptide in 14 of 17 positions, with two 6-residue stretches of exact identity (Figure 1B). Other oligonucleotide probes designed from GP IIb peptides did not detect positive plaques in the HUVE cell cDNA library. While this work was in progress, a partial cDNA sequence was reported for the carboxyl-terminal region of FnR_α (Argraves et al., 1986). The published sequence is

[2] L. Fitzgerald and D. R. Phillips, unpublished observations.

FIGURE 1: Selection of FnR_α cDNA clones using an oligonucleotide probe designed from a peptide of platelet GP IIb. (A) Nucleotide sequence of the 51-mer oligonucleotide probe vs the FnR_α cDNA nucleotide sequence. (B) Amino-terminal protein sequence of the GP IIb cyanogen bromide fragment vs the translation of the probe-selected cDNA clone. Identities are indicated by vertical bars. The methionine (M) in the peptide sequence was deduced from cyanogen bromide cleavage.

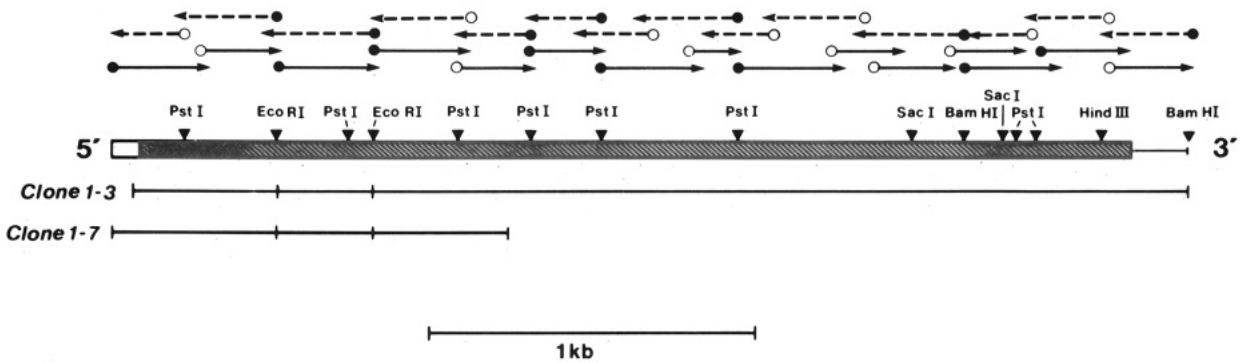

FIGURE 2: Diagram of the FnR_α cDNA clones (1–3, 1–7) showing relevant restriction sites and the sequencing strategy.

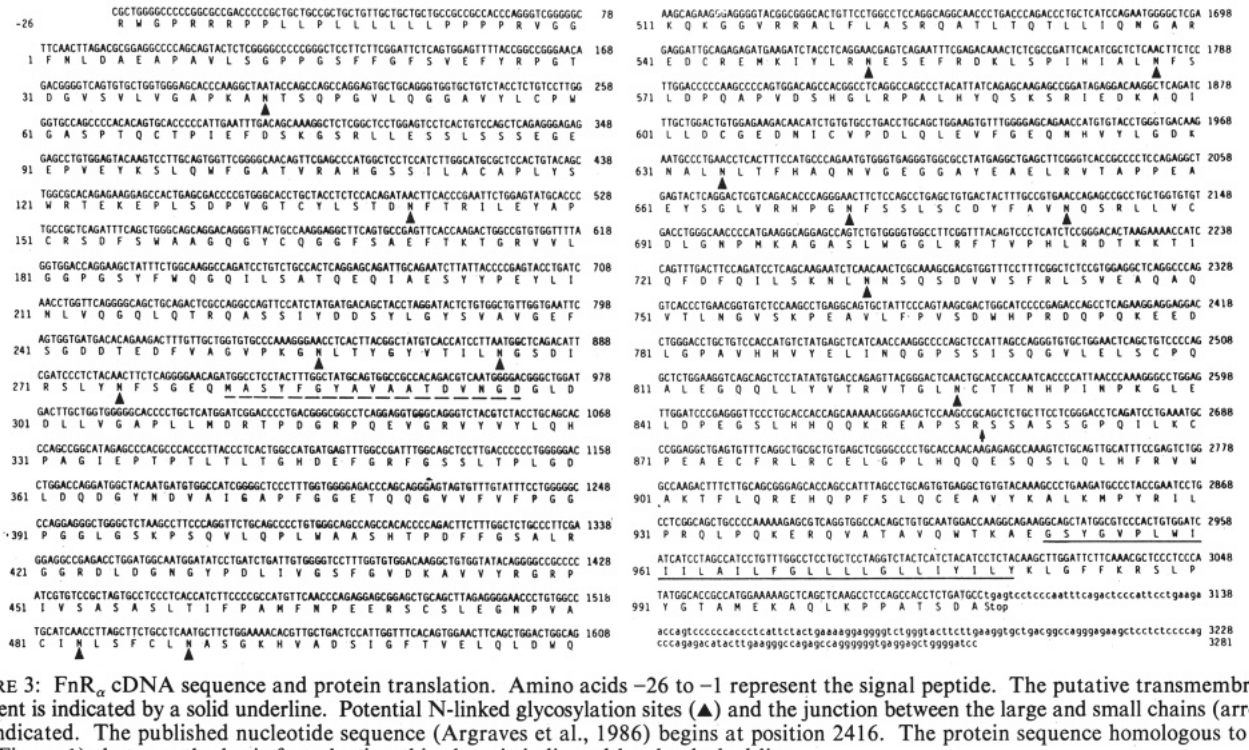

FIGURE 3: FnR_α cDNA sequence and protein translation. Amino acids −26 to −1 represent the signal peptide. The putative transmembrane segment is indicated by a solid underline. Potential N-linked glycosylation sites (▲) and the junction between the large and small chains (arrow) are indicated. The published nucleotide sequence (Argraves et al., 1986) begins at position 2416. The protein sequence homologous to GP IIb (Figure 1) that was the basis for selecting this clone is indicated by the dashed line.

identical with that of the HUVE cDNA clone for 866 nucleotides at the 3′ end.

A restriction map and the sequencing strategy used for the two overlapping FnR_α cDNA clones are shown in Figure 2. The 3281-base cDNA sequence was obtained on both strands. Figure 3 shows the cDNA sequence and its translation, which resulted in a single open-reading frame including the entire mature protein (1008 amino acids) and part of the signal peptide (26 amino acids). The cDNA sequence includes only 179 nucleotides of the 3′ untranslated region, which has been reported to be 1048 nucleotides long (Argraves et al., 1986). The peptide sequence of the amino terminus of the FnR_α has not been reported; it was assigned from the cDNA translation on the basis that the α-subunits of the adhesion receptor family have an almost invariant Asn-Leu-Asp sequence in positions 2–4 (Charo et al., 1986; Suzuki et al., 1986; Takada et al., 1987b). The derived amino acid sequence (Figure 3, −26 to −1) 5′ to the predicted amino terminus contains primarily hydrophobic residues, a characteristic of signal peptides. Analysis of the FnR_α protein sequence by the method of Kyte and Doolittle (1982) using a windowing average of 15 residues showed a single, strongly hydrophobic stretch from residues 952 through 980 (Figure 3). This region probably represents the transmembrane domain. Thus, over 90% of the FnR_α is extracellular, the putative cytoplasmic domain having only 28 residues. The FnR_α consists of one large and one small chain, which are disulfide linked (Argraves et al., 1986; Hynes, 1986). Both of these chains are encoded by a single mRNA, and the propeptide is believed to be cleaved between the arginine and serine residues at positions 858 and 859 (Argraves et al., 1986;

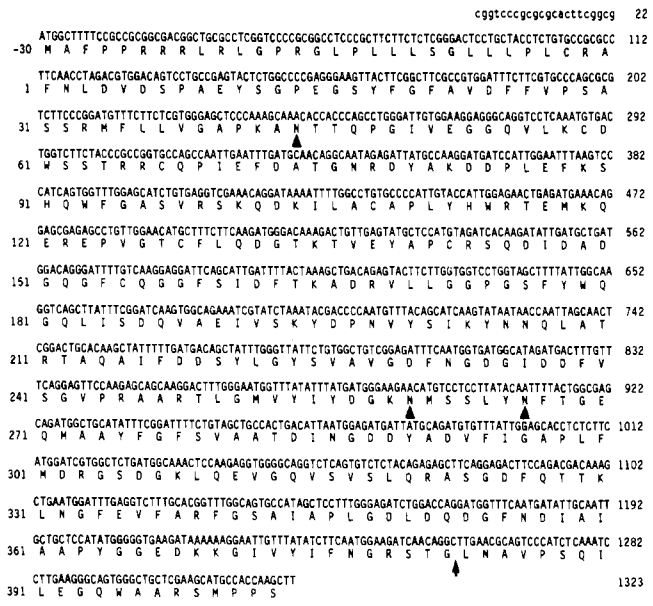

FIGURE 4: VnR_α 5′ cDNA sequence and protein translation. Amino acids −30 to −1 represent the signal peptide. The potential N-linked glycosylation sites are indicated (▲). The published sequence (Suzuki et al., 1986) begins at nucleotide position 1257, and translation starts at the point indicated by the arrow.

Suzuki et al., 1986). Thus, the FnR_α consists of a large chain of 858 amino acids and a small chain of 150 amino acids. The FnR_α contains 14 potential N-linked glycosylation sites having the sequence Asn-X-Ser/Thr, all of which are in the large subunit (Figure 3). Thus, the difference between the cDNA-predicted molecular weight for the large chain (92 941) and the molecular weight determined from SDS gels (140 000) (Pytela et al., 1986) can be largely accounted for by the number of potential glycosylation sites. The reason for the difference between the cDNA-predicted molecular weight of the small chain (17 088) and that estimated from SDS gels (25 000) is not clear.

A HUVE cell cDNA clone specific for the VnR_α was obtained by using an oligonucleotide probe made to a region of the recently published partial cDNA sequence for this protein (Suzuki et al., 1986). Figure 4 shows the cDNA sequence and protein translation for the 5′ region of the VnR_α, which contains the amino-terminal 382 amino acids, a putative 29 amino acid signal peptide, and 22 nucleotides of the 5′ untranslated sequence, all of which were missing in the published sequence. Sixty-seven nucleotides that overlap the published sequence and a 240-base segment 5′ to the first *Sac*I site (data not shown) were also identified; they were identical with those of the published sequence. When the cDNA sequence in the present report is combined with the published sequence, the deduced sequence of VnR_α contains 1018 amino acids: 860 in the large chain and 158 in the small chain. The VnR_α has 13 potential N-linked glycosylation sites: 10 in the large chain and 3 in the small chain. The cDNA-deduced amino terminus of the large chain is identical with the published amino acid sequence (Suzuki et al., 1986).

The cDNAs corresponding to platelet GP IIb were isolated from a HEL cell cDNA expression library by antibody screening (Poncz et al., 1987). These cDNAs contain the entire GP IIb protein-coding sequence, including 1008 amino acids for the mature protein and 30 amino acids representing a signal peptide. The GP IIb large chain contains 871 amino acids, and the small chain contains 137 amino acids. The predicted GP IIb protein sequence contains the partial peptide sequences obtained from the amino termini of the large and small chains of purified platelet GP IIb (Charo et al., 1986). In addition, the 17 amino acids used to design the oligonucleotide probe shown in Figure 1 are contained in positions 285–301 of the translated GP IIb sequence (Figure 5). These sequence identities confirm that the GP IIb like glycoprotein synthesized and expressed by HEL cells is the same gene product as that found in platelets. There are five potential N-linked glycosylation sites in GP IIb: four in the large chain and one in the small chain.

Table I: Protein Sequence Identities in the Three α-Subunits[a]

	large chain		small chain		overall	
	no.	%	no.	%	no.	%
common	261	28.9	27	17.0	288	27.1
FnR_α vs VnR_α	424	47.0	49	30.8	473	44.5
FnR_α vs GP IIb	359	39.8	43	27.1	402	37.9
VnR_α vs GP IIb	340	37.7	43	27.0	383	36.1

[a] Calculated from data shown in Figure 5. To determine percentages, the numbers were derived by the total of positions in the alignment, including gaps.

An alignment of the cDNA-predicted protein sequences of the large and small chains of the three proteins is shown in Figure 5. The best alignment was obtained when the cysteines were placed in register. The sizes of the large and small chains in the three proteins differ slightly. Table I lists the overall identities in protein sequence. A majority (288 positions) of the amino acid identities are conserved in all three proteins. The FnR_α and VnR_α are the most homologous, while GP IIb and the VnR_α are the least homologous. The large chains are more homologous than the small chains. The region of greatest similarity among all three α-subunits extends from positions 224–436 (FnR_α); 93 identical positions (43.7%) are found in this region. Each protein in this region has four repeated segments (Figure 5) of about 30 amino acids. Within each segment is a stretch of 12 amino acids that contains Asp, Asn, and hydrophobic residues in a spacing that resembles the β-turns of other Ca^{2+}-binding proteins (Kretsinger & Nockolds, 1973; Gariépy & Hodges, 1983). Figure 6 shows a representative Ca^{2+}-binding repeat of the FnR_α and bovine brain calmodulin. This Ca^{2+}-binding sequence of calmodulin has the characteristic "EF hand" structure: the Ca^{2+}-binding coordinates are located in a β-turn, which is flanked by α-helical regions. In contrast, these α-helices are missing from the putative Ca^{2+}-binding segments of the adhesion receptor. Overall, the four repeated putative Ca^{2+}-binding regions in the three α-subunits contain about 109 residues (FnR_α), 62 of which (57%) are conserved. The four repeats are separated from each other by stretches of about 30 amino acids of lower homology. Outside of the putative Ca^{2+}-binding region, identical positions are generally clustered into stretches of 4–10 residues. The carboxyl-terminal region of the large chain (positions 837–858 of FnR_α) and the amino-terminal region of the small chain (positions 1–59 of FnR_α) have a very low degree of similarity (8.5%). In all three proteins, an arginine residue has been assigned to the carboxyl terminus of the large chain. The amino-terminal sequences of the VnR_α (Suzuki et al., 1986) and GP IIb small chains (Charo et al., 1986) have been determined, and the translation of the cDNAs for these two proteins places this arginine residue in the adjacent position. The amino-terminal sequence of the FnR_α small chain has not been reported, but on the basis of sequence homology, we have assigned the arginine at position 858 as the carboxyl terminus of its large chain.

The three adhesion receptor α-subunit proteins differ in their number of cysteines. The FnR_α has 20 cysteines: 16 in the

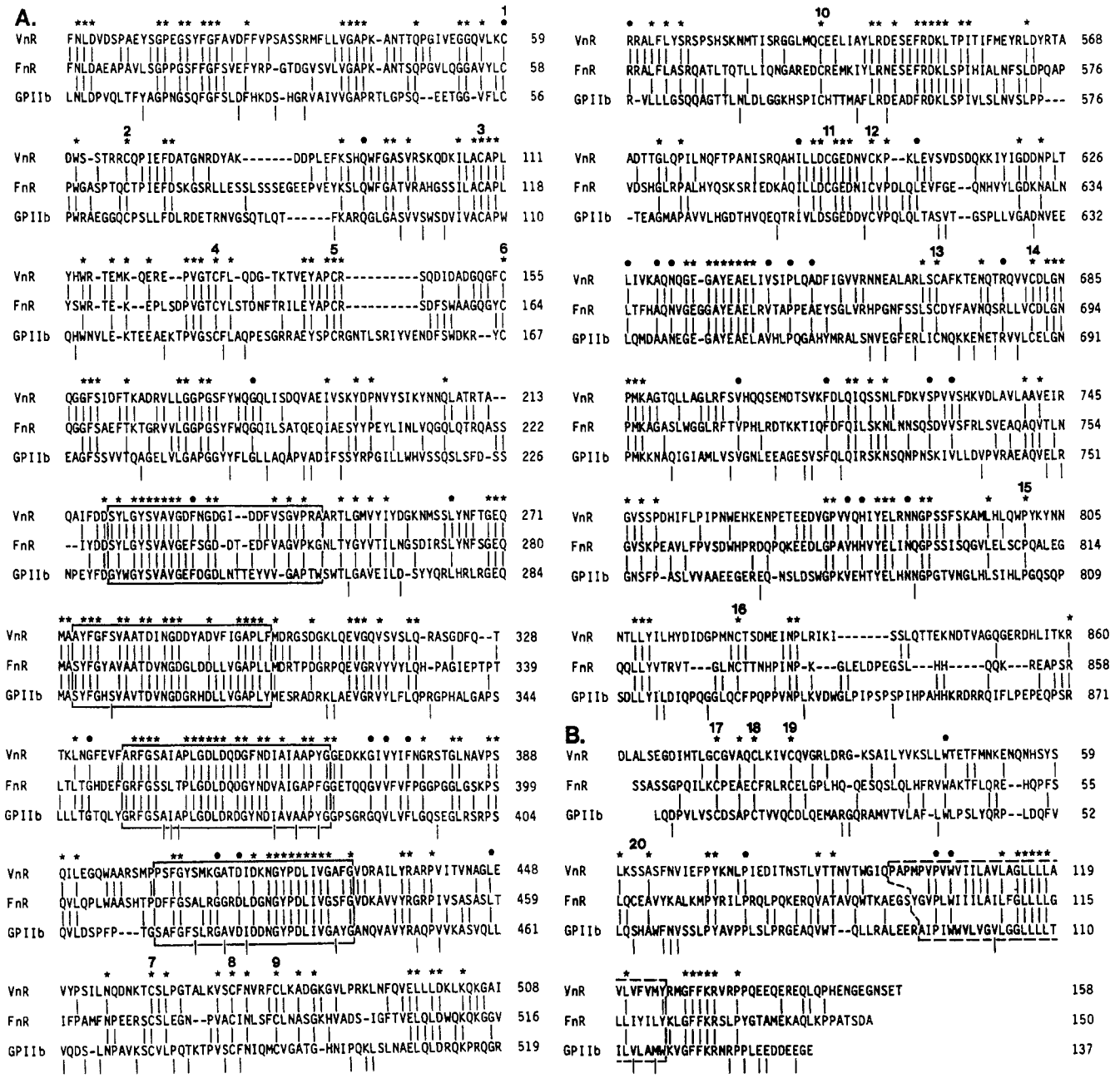

FIGURE 5: Alignment of the cDNA-deduced protein sequences of the VnR (top rows) and FnR (middle rows) α-subunits and of GP IIb (bottom rows). The VnR_α protein sequence starting at position 404 is taken from the published sequence (Suzuki et al., 1986). Amino acids at an identical position in all three proteins are indicated at the top (asterisks), and those at an identical position in the VnR_α and GP IIb are indicated at the bottom of each set of rows (vertical bar). (A) Large chains. (B) Small chains. Cysteines for the FnR_α are numbered consecutively. The four putative Ca^{2+}-binding domains are boxed with solid lines, and the transmembrane segments are boxed with dashed lines.

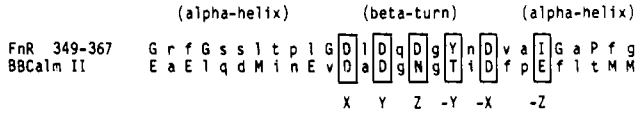

FIGURE 6: Amino acid sequences illustrating the putative Ca^{2+}-binding sequences for the adhesion receptor α-subunits and the "EF hand" structure. The Ca^{2+}-binding coordinates are shown on the bottom, and the corresponding residues are boxed. BBCalm II is bovine brain calmodulin, second binding repeat (Gariépy & Hodges, 1983). Secondary structure is shown at the top. Residues indicated by upper-case letters in FnR in the α-helical regions show positions not favoring α-helix, while the upper-case letters in the BBCalm sequence indicate residues favoring α-helix.

large chain and 4 in the small chain. If these are numbered consecutively, then the VnR_α is missing cysteines 15 and 20, while GP IIb is missing cysteines 11, 15, and 20. All three proteins have six cysteines in the amino-terminal region of the large chain, whereas the region of the putative Ca^{2+}-binding repeats contains no cysteines. The large and small chains of GP IIb are apparently joined by a single interchain disulfide, since incubation of platelet GP IIb–IIIa with an increasing concentration of β-mercaptoethanol results in a separation of the large and small chains of GP IIb, followed by an increase in molecular weight of the small chain.[2] Thus, two of the three cysteines in the GP IIb small chain are in the form of an intrachain disulfide, which leaves only a single cysteine available to form the interchain disulfide. If all of these proteins have a single interchain disulfide, then free sulfhydryls may be present in the large and small chains of FnR_α and the large chain of GP IIb.

DISCUSSION

The presence of endothelial cell membrane proteins related to the platelet GP IIb–IIIa complex has been established independently in a number of laboratories (Fitzgerald et al.,

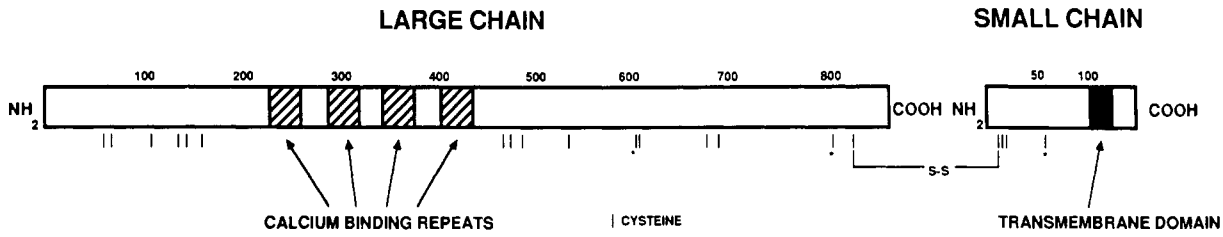

FIGURE 7: Predicted structure of the two-chain α-subunits of the adhesion receptor family. The asterisks below certain cysteines indicate the cysteines absent from the VnR_α, GP IIb, or both (see Figure 5).

1985b; Thiagarajan et al., 1985; Leeksma et al., 1986; Newman et al., 1986; Plow et al., 1986b). Recently, we reported that the primary amino acid sequences of GP IIIa in platelets and HUVE cells are identical (Fitzgerald et al., 1987). However, cDNAs identified by using an oligonucleotide probe designed from the sequence of a platelet GP IIb peptide corresponded to that of the FnR_α. Subsequently, probes based on the GP IIb cDNA sequence of HEL cells detected no positive clones. Recently, it was reported that GP IIIa and the β-subunit of the VnR are immunologically similar and have identical amino-terminal peptide sequences (Ginsberg et al., 1987). In this report, we have demonstrated the presence of VnR_α clones in a HUVE cell library. Thus, it appears that the previously described endothelial cell GP IIb–IIIa like proteins are, in fact, the VnR.

The terms "cytoadhesins" (Plow et al., 1986b) and "integrins" (Hynes, 1987) have been proposed to describe the adhesion receptor family, which consists of α/β-subunit heterodimers. The three β-subunits, which define the three subfamilies, are of similar size (769–803 amino acids long) (Tamkun et al., 1986; Fitzgerald et al., 1987; Kishimoto et al., 1987), and they have 56 cysteines, 4 cysteine-rich tandem repeats, a carboxyl-terminal transmembrane segment, a short cytoplasmic domain, and a highly homologous region from amino acids 110 to 350. These heterodimer receptors mediate cell adhesion by interacting primarily through Arg-Gly-Asp sequences present on their ligands (Ruoslahti & Pierschbacher, 1986; Wright et al., 1987). The GP IIb–IIIa complex and the VnR can be purified by affinity chromatography using immobilized Arg-Gly-Asp-containing peptides (Pytela et al., 1986), and Arg-Gly-Asp-containing peptides will inhibit cell adhesion (Ruoslahti & Pierschbacher, 1986) and platelet aggregation (Gartner & Bennett, 1985; Haverstick et al., 1985). Leukocyte recognition of C3bi-containing particles, mediated by the Mac-1 receptor, also appears to occur by an Arg-Gly-Asp-dependent process (Wright et al., 1987).

The FnR has been identified in human and hamster cell lines by affinity chromatography (Pytela et al., 1985a) and by using polyclonal and monoclonal antibodies (Brown & Juliano, 1985, 1986). The mammalian FnR is often referred to as the 140-kDa complex because both the α- and β-subunits have molecular weights of 140000 on reduced SDS–polyacrylamide gels. The mammalian FnR is specific for fibronectin (Pytela et al., 1986), while a homologous three-protein complex (bands 1–3) in chickens, the CSAT antigen, can bind both fibronectin (Horwitz et al., 1985; Akiyama et al., 1986) and laminin (Horwitz et al., 1985). The VnR was first described in osteosarcoma cells by affinity chromatography on either vitronectin–Sepharose or Sepharose linked to a synthetic peptide containing the Arg-Gly-Asp sequence (Pytela et al., 1985b). The GP IIb–IIIa complex is a fibrinogen, fibronectin, and von Willebrand factor receptor required for the aggregation of stimulated platelets (Plow et al., 1986a).

The present study establishes the structure of FnR_α, one of the α-subunits of the adhesion receptor family. Our identification of the cDNA-derived sequence as the FnR_α depends on three lines of evidence. First, a published partial cDNA sequence for a FnR α-subunit (Argraves et al., 1986) is identical for 866 nucleotides with the cDNA sequence in the present report. Second, the amino-terminal sequences of the five VLA have been reported (Takada et al., 1987b). The amino-terminal sequence of the α-subunit of VLA-5 (Takada et al., 1987b), which is thought to be the FnR, matches the predicted sequence shown in Figure 3. Third, we have detected the presence of the 140-kDa FnR complex in HUVE cells by using polyclonal antibodies.[3]

The three α-subunits have several structural features in common (Figure 7). The proteins consist of two disulfide-linked polypeptide chains (Argraves et al., 1986; Suzuki et al., 1986; Poncz et al., 1987), and the cleavage of the propeptide appears to occur at an Arg residue (Argraves et al., 1986; Poncz et al., 1987). All three α-subunits have a single transmembrane segment, which is located near the carboxyl terminus of the small chain. This location leads to the prediction of a short cytoplasmic domain of 20–32 residues. The extracellular large chains are linked by a single interchain disulfide bond to the small chains. The three α-subunits are homologous (Suzuki et al., 1986; Poncz et al., 1987); the alignment of their protein sequences results in overall identities of 36.1–44.5%. Identical residues appear clustered, most notably in the four repeated segments that contain sequences of amino acids characteristic of Ca^{2+}-binding proteins (Poncz et al., 1987).

The putative Ca^{2+}-binding sequences of the adhesion receptor α-subunit differ from those of other Ca^{2+}-binding proteins in three respects. First, the α-helices before and after the Ca^{2+}-binding β-turns in proteins such as calmodulin, troponin C, and parvalbumin (Gariépy & Hodges, 1983), i.e., the thumb and forefinger of the so-called EF hand (Kretsinger & Nockolds, 1973), appear to be absent from the putative Ca^{2+}-binding sites of the adhesion receptor α-subunits. The glycine and proline residues in these regions (Figure 6) have a low probability of forming α-helices as analyzed by the Chou and Fasman (1978) rules. Second, the −Z positions of the Ca^{2+}-binding sites of calmodulin, troponin C, and parvalbumin are invariably occupied by Glu, most likely because of its strong helix-forming potential. In the adhesion receptor α-subunits, however, these positions are occupied by Val or Ile, which does not form strong helices. Third, the spacing between the putative Ca^{2+}-binding sites in the α-subunits of the adhesion receptors (43–55 residues) differs from those of other Ca^{2+}-binding proteins (18–28 residues). The importance of Ca^{2+} is best understood for the platelet GP IIb–IIIa complex. Chelation of Ca^{2+} causes dissociation of GP IIb–IIIa, either in detergent solutions (Kunicki et al., 1981) or on intact platelets (Fitzgerald & Phillips, 1985). The role of Ca^{2+} in the structure and function of other adhesion receptor complexes has not been determined; however, it is known that the VnR

[3] I. Charo, L. Bekeart, and D. R. Phillips, unpublished observations.

of HUVE cells cannot be dissociated by Ca^{2+} chelation (Fitzgerald et al., 1985b; Leeksma et al., 1986).

The protein sequence homologies among the α- and β-subunits of the adhesion receptor family suggest that they evolved by gene duplication. Alignment of the sequences of the β-subunits showed that the chicken FnR/laminin receptor band 3 (equivalent to the human FnR_β and VLA_β) has a higher homology to GP IIIa and the leukocyte β-subunit (45.4%) than does GP IIIa to the leukocyte β-subunit (38.5%).[2] Similarly, the FnR_α has higher homology to GP IIb and the VnR_α than does GP IIb to the VnR_α (Table I). This indicates that the precursors to the FnR α- and β-subunits could have given rise to the other adhesion receptor subunits. The subfamily of adhesion receptors defined by the FnR/VLA β-subunit appears to be more diverse than the other two subfamilies; this subfamily has at least five separate α/β-heterodimers, and its α-subunits consist of both single-chain and two-chain proteins (Hynes, 1987).

A group of *Drosophila* proteins, termed "position-specific antigens", resembles the adhesion receptors. They have an α/β-heterodimeric structure, with subunits of M_r 110000–150000 (Wilcox & Leptin, 1985; Leptin, 1986). The position-specific antigens have unique α-subunits complexed with a common β-subunit, and their role in embryogenesis may involve cell migration and adhesion. The amino-terminal sequence of one of the *Drosophila* α-subunits is homologous to the adhesion receptor α-subunits (Takada et al., 1987b).

The cDNA-deduced protein sequence for several α- and β-subunits offers the possibility of a greater understanding of their functional roles as mediators of cell adhesion and platelet aggregation. Studies to determine the locations of ligand recognition sites, the regions involved in the noncovalent association of the subunits, and the sites of possible interaction with the cytoskeleton will elucidate the relative importance of conserved and divergent regions that regulate the common and the specific functions in this receptor family.

ACKNOWLEDGMENTS

We thank Dr. Stuart Orkin of Harvard Medical School and Dr. David Ginsberg of The University of Michigan for providing the HUVE cell cDNA library, Drs. John Taylor, Brian McCarthy, and Beatriz Levy-Wilson for help in planning this study, Drs. Brian Blackhart and Stephen Lauer for advice on experimental procedures, Erwin Ludwig, Vince Pierotti, and Richard Otter for advice on DNA sequencing and sequence analysis, Kate Sholly and Theresa Cardinale for manuscript preparation, James X. Warger for graphics and photography, and Sally Gullatt Seehafer and Al Averbach for editorial assistance.

Registry No. Receptor, fibronectin (human clone 1-7/1-3 α-subunit heavy chain protein moiety reduced), 111237-06-0; receptor, fibronectin (human clone 1-7/1-3 α-subunit light chain protein moiety reduced), 111237-07-1; receptor, vitronectin (human α-subunit heavy chain protein moiety reduced), 111237-08-2; glycoprotein IIb (human HEL cell heavy chain protein moiety reduced), 111237-04-8; glycoprotein IIb (human HEL cell light chain protein moiety reduced), 111237-05-9; calcium, 7440-70-2.

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Crystal Structures of Metyrapone- and Phenylimidazole-Inhibited Complexes of Cytochrome P-450$_{cam}$†

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ABSTRACT: The crystal structures of metyrapone- and 1-, 2-, and 4-phenylimidazole-inhibited complexes of cytochrome P-450$_{cam}$ have been refined to a nominal resolution of 2.1 Å and compared with the 1.63-Å camphor-bound structure. With the exception of 2-phenylimidazole, each of the inhibitors forms an N–Fe bond with the heme iron atom while part of the inhibitor sits in the camphor-binding pocket. In the 2-phenylimidazole complex, a water molecule or hydroxide ion coordinates with the heme iron atom while the inhibitor binds in the camphor pocket adjacent to the aqua ligand. Each of the inhibitors forces the central region of helix I that forms part of the O_2 binding pocket to move away from the inhibitor, with the exception of 2-phenylimidazole where the helix moves in toward the inhibitor. In addition, the Tyr-96 region, which provides specific contact points with the substrate, is perturbed, although to varying degrees with each inhibitor. These perturbations include large, localized changes in Debye–Waller or temperature factors, indicative of changes in dynamical fluctuations. The largest inhibitor, metyrapone, causes the fewest changes, while 2-phenylimidazole binding causes the largest, especially in helix I. The large 2-phenylimidazole-induced movement of helix I can be rationalized on the basis of the inhibitor imidazole group's hydrogen-bonding requirements.

Cytochromes P-450 are a widely occurring class of *b*-type heme proteins that catalyze the hydroxylation of both aliphatic and aromatic molecules (Hayaishi, 1974). Much of what is known about the details of cytochrome P-450 structure and function stems from studies with cytochrome P-450$_{cam}$ obtained from the soil bacteria *Pseudomonas putida* (Wagner & Gunsalus, 1982; Gunsalus et al., 1974; Debrunner et al., 1978; Gunsalus & Sligar, 1978). P-450$_{cam}$ catalyzes the conversion of camphor to 5-*exo*-hydroxycamphor (Gelb et al., 1982) and is the first step in the oxidative assimilation of camphor when camphor is utilized by *P. putida* as a carbon source (Gunsalus et al., 1974).

The crystal structure of P-450$_{cam}$ has been determined with substrate bound first at 2.6 Å (Poulos et al., 1985) and then 1.63 Å (Poulos et al., 1987). In addition, the substrate-free structure has been refined at 2.1 Å (Poulos et al., 1986). The substrate, camphor, contacts the heme ring immediately adjacent to the oxygen-binding site and is held in place by complementary contacts with neighboring aliphatic and aromatic residues in addition to a single hydrogen bond between the camphor carbonyl oxygen atom and the hydroxyl group of Tyr-96. When camphor is bound, the high-spin heme iron atom is pentacoordinate with the sulfur atom of Cys-357 serving as the only axial ligand (Poulos et al., 1985). In the absence of camphor, the substrate pocket fills with a hydrogen-bonded array of solvent molecules, one of which coordinates with the iron atom (Poulos et al., 1986), giving a hexacoordinate, low-spin heme. No significant conformational

† This work was supported in part by NIH Grant GM 33688.
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0006-2960/87/0426-8165$01.50/0