Molecular Cloning of the Rat Integrin α_1-Subunit: A Receptor for Laminin and Collagen

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Abstract. Integrin heterodimers mediate a variety of adhesive interactions, including neuronal attachment to and process outgrowth on laminin. We report here the cloning and primary sequence of an M_r-200 kD integrin α subunit that associates with the integrin β_1 subunit to form a receptor for both laminin and collagen. Similarities in ligand-binding specificity, relative molecular mass and NH_2-terminal sequence make this a strong candidate for the rat homologue of the α subunit of the human integrin VLA-1. The full-length rat α_1 cDNAs encode a protein containing a putative signal sequence and a mature polypeptide of 1,152 amino acids, with extracellular, transmembrane and cytoplasmic domains. Several structural features are conserved with other integrin α chains, including (*a*) a sequence motif repeated seven times in the NH_2-terminal half; (*b*) potential Ca^{2+}/Mg^{2+} binding sites in repeats 5, 6, and 7, and (*c*) alignment of at least 14 of 23 cysteine residues. This rat α_1 sequence also contains a 206-amino acid I domain, inserted between repeats 2 and 3, that is homologous to I domains found in the same position in the alpha subunits of several integrins (VLA-2, Mac-1, LFA-1, p150). The rat α_1 and human VLA-2 α subunits share >50% sequence identity in the seven repeats and I domain, suggesting that these sequence identities may underlie some of their similar ligand-binding specificities. However, the rat integrin α_1 subunit has several unique features, including a 38-residue insert between two Ca^{2+}/Mg^{2+} binding domains, and a divergent 15-residue cytoplasmic sequence, that may potentially account for unique functions of this integrin.

LAMININ (LN),[1] a prominent component of the extracellular matrix, is an adhesive glycoprotein that has potent effects on many cells. Neuronal process outgrowth, substratum adhesion, migration, survival, and differentiation are all promoted by LN (see Sanes, 1989 for review). Outside of the nervous system, LN is also a major constituent of basement membranes, where it promotes the adhesion, growth, migration, and differentiation of many cell types (see Beck et al., 1990). Adhesion of cells to LN is divalent cation dependent (Turner et al., 1987), and is blocked by anti-integrin (anti-β_1) antibodies (Bozyczko and Horwitz, 1986; Tomaselli et al., 1987).

Integrins are dimers of α and β subunits that mediate cell–cell as well as cell–matrix adhesion (see Hemler, 1990; Kishimoto et al., 1989; Ginsberg et al., 1988 for review). At least 5 distinct β subunits and 11 different α subunits have been identified. Individual α and β subunits form heterodimers with their own specific binding properties. The major classes, determined at present by their shared β subunits include: (*a*) six very late antigen (VLA) proteins that associate with the β_1 subunit (Hemler, 1990) (*b*) three leukocyte antigens, LFA-1, Mac-1, and p150, that associate with the β_2 subunit (Kishimoto et al., 1989) and (*c*) GPIIb and the vitronectin receptor α subunit that associate with the β_3 subunit (Ginsberg et al., 1988).

Recent work has identified at least two and possibly as many as four new β subunits (Kajiji et al., 1989; Cheresh et al., 1989; Holzmann and Weissman, 1989; Freed et al., 1989). Several candidates for additional α subunits have also been identified (Bourdon and Ruoslahti, 1989; Kramer and Marks, 1989). Individual α subunits have also been found paired with different β subunits (see Cheresh et al., 1989; Kajiji et al., 1989; Hemler et al., 1989) with potentially varied ligand affinities. Therefore, it is feasible that a large number of α and β subunits can combine to generate additional combinations of heterodimers with novel binding properties. In addition the ligand binding specificity of individual integrin heterodimers can be modified by cell-specific factors (Languino et al., 1989; Elices and Hemler, 1989) engendering additional functional diversity within the integrin superfamily.

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1. *Abbreviations used in this paper*: LN, laminin; VLA, very late activation.

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Both affinity chromatography of receptors in solution and antibody perturbation studies on intact cells have provided evidence that human and rat $\alpha_1\beta_1$ integrins function as receptors for LN and collagen. Using human JAR cells, an anti α_1-mAb has been shown to inhibit cell binding to LN (Hall et al., 1990). On LN, this antibody inhibits interactions with a fragment E1, containing the core and proximal portions of the short arms of LNs cruciform structure. A similar mAb, 3A3, specific for an M_r-200 kD rat integrin α subunit has been shown to inhibit interactions of PC12 pheochromocytoma cells with collagen and LN (Turner et al., 1989). As observed with the anti-human α_1 mAb, the rat-specific mAb, 3A3, inhibits interactions with the fragment E1 containing the core and portions of the short arms of LN (Tomaselli, K. T., and L. F. Reichardt, personal communication). The antigen defined by the 3A3 mAb appears to correspond to the integrin α subunit of a LN receptor purified from neural tissue by affinity chromatography on whole LN (Ignatius and Reichardt, 1988). Immunoaffinity purification of the 3A3 antigen from neonatal rat tissues and microsequencing suggest further that this protein is homologous to the human α_1 subunit (Tawil et al., 1990). Therefore, the similarities in ligand-binding specificities, α subunit relative molecular mass, and microsequencing data indicate that the rat α subunit defined by the 3A3 mAb is the rat homologue of the human α_1 subunit.

Whereas many integrin receptors share a common ligand, like LN, it is becoming apparent that each has its own unique ligand specificities and binding properties that mediate distinct cellular responses. Because receptors with different ligand affinities share an identical β subunit, specificity must depend in part on divergent structures within the individual αs. Thus, comparison of the primary structures of αs with defined ligand properties may reveal unique domains related to ligand specificity and affinity.

In this paper, we describe the cloning and sequence analyses of the rat integrin α subunit recognized by the 3A3 mAb. Our data provide further evidence that this is the rat homologue of the human VLA-1 α subunit. Analysis of the sequence reveals several structures shared by other integrins, including (*a*) an inserted or "I" domain; (*b*) seven noncontiguous repeats and (*c*) three highly conserved metal binding domains. Between two of these conserved metal binding domains is a 38-residue, nonconserved segment containing four cysteines. This domain, along with surrounding conserved cation binding sites, possibly interacting with associated β structures, may form a portion of a novel ligand binding site for this $\alpha_1\beta_1$ dimer.

Materials and Methods

Reagents and Solutions

Nitrocellulose filters used for filter lifts were from Schleicher & Schuell, Inc. (Keene, NH). Restriction enzymes, Klenow fragment of DNA polymerase and T4 polynucleotide kinase were from New England Biolabs (Beverly, MA) and Boehringer Mannheim Diagnostics (Houston, TX). Exonuclease III and other enzymes used to generate unidirectional deletions of inserts for sequencing were from Promega Biotec (Madison, WI) and used according to manufacturer's recommendations. *Thermus aquaticus* polymerase (TAQ) used in all polymerase chain reactions was from Cetus Corp. (Emeryville, CA). Reagents used for sequencing, including sequenase enzyme, and hexanucleotide primers were from kits supplied by U. S. Biochemical Corp. (Cleveland, OH). [α-^{35}S]dATP, [γ-^{32}P]ATP, and [α-^{32}P]dCTP were from Amersham Chemical Co. (Arlington Heights, IL). Other chemicals not specified were purchased from Sigma Chemical Co. (St. Louis, MO).

Denhardt's solution (1×) is 0.02% polyvinyl pyrrolidone, 0.02% BSA, with 0.02% Ficoll Type 400. Hybridization buffer is 900 mM sodium chloride, 5 mM EDTA, 50 mM sodium phosphate (pH 7.4), 5× Denhardt's solution, 20% formamide, and 100 μg/ml salmon sperm DNA. 1× SSC is 150 mM sodium chloride and 15 mM sodium citrate (pH 7.0).

Screening of cDNA Library

A rat pheochromocytoma, PC12, cDNA library in lamda ZAP was obtained from Dr. Jim Boulter and Dr. Steve Heinemann. The library was plated on *Escherichia coli* strain Y1090, and replica filters were prepared according to established procedures (Maniatis et al., 1989). Filters were screened with an end labeled, nondegenerate, 48-base oligonucleotide (CTG CAC TGT GTA GCC AAA CAT GTC CTC CAC AGG GCC AGA GAA GGA CAT). This oligonucleotide corresponds to positions 10–24 of the amino acid sequence derived from antibody purified protein. Strategy for selection of individual codon choices were according to Lathe (1985). Hybridizations and wash conditions, with a determined mismatch of 72% from the chosen oligonucleotide and the derived rat α_1 sequence, were for low and high stringency, calculated to be 19°C and 5°C below the melting temperature of duplex DNA, respectively (Thomas and Dancis, 1973; Bonner et al., 1973).

Positive plaques from the oligonucleotide screen were further screened by polymerase chain reaction (PCR) using two synthetic oligonucleotides: a 20-mer corresponding to amino acids 1–7 (256-fold degenerate) and a 21-mer from amino acids 18–24 (512-fold degenerate). Each primer contained an additional eight nucleotides encoding an Eco RI site for subcloning of the products for sequencing. A product of the expected size, 88 bp, was interpreted as strong evidence that a particular clone contained the desired insert. To confirm the identity of the clones, the PCR products were subcloned into m13mp18 and sequenced.

Insert-containing plasmid derived from lamda ZAP were then isolated by coinfection with the helper phage M13K07. The plasmid DNA was used to transform *E. coli* strain, BB4. Transformed colonies, selected for ampicillin resistance, were then coinfected with the same helper phage to release phage containing single-stranded DNA for sequencing.

DNA Sequencing

Nucleotide sequence was determined from two independent clones containing the entire coding region inserted in opposite orientations in Bluescript SK$^-$. Sequencing was according to the dideoxy chain termination method (Sanger et al., 1977) using both unidirectional deletions achieved with exonuclease III digestion and by extension on undigested clones with synthetic primers.

Nucleic Acid and Amino Acid Sequence Analysis

Nucleic acid and amino acid sequence analysis were performed with the PCGENE package of programs (Intelligenetics Corp., Mountain View, CA). Signal sequence cleavage analysis was according to von Heijne (1986), hydrophobicity plots according to Kyte and Doolittle (1982). Multiple sequence alignments were according to Sobel and Martinez (1985) and where necessary manually edited to align cysteine residues.

RNA Analysis

Poly A+ RNA from PC12 cells grown on culture dishes was prepared by oligo dT column chromatography after isolation of total RNA on CsCl gradients. RNA blots were prepared using standard methods as described by Maniatis et al. (1989) using cDNA probes radiolabeled by random priming (Feinberg and Vogelstein, 1984) using hexanucleotides purchased from Pharmacia Fine Chemicals (Piscataway, NJ).

Results

An mAb 3A3, which immunoprecipitates an M_r-200 kD integrin α subunit in association with the integrin β1 subunit and blocks the binding of several rat cell types to LN and collagen (Turner et al., 1989), was used to purify sufficient

Comparison with Integrin α subunit N-terminal sequences

	1								10					15
deduced rat alpha sequence	F	N	V	D	V	K	N	S	M	S	F	S	G	P	V
human alpha 1	-	-	-	-	-	-	D	-	-	T	-	L	-	-	-

Homology to other β_1 associated alpha chains

Number of identical amino acids	α_1	α_2	α_3	α_4	α_5	α_6
	12/15	6/15	3/14	6/14	5/15	3/15

Figure 1. Comparison of NH_2-terminal sequence derived from protein purified with the mAb 3A3 with NH_2-terminal sequences of human β_1 family alpha subunits. NH_2-terminal sequence information for human VLA subunits α_1 and α_3 are according to Takada et al. (1987); information for subunit α_4 is according to Takada et al. (1989); information for subunit α_2 is according to Takada and Hemler (1989); and information for subunit α_5 or the fibronectin receptor alpha subunit is according to Argraves et al. and Fitzgerald et al. (1987). Hyphens designate identical residues in the human VLA alpha subunits while nonmatching residues are shown.

amounts of protein from whole rat neonates, for NH_2-terminal sequencing (Tawil et al., 1990). As shown in Fig. 1, the partial sequence of the protein reveals extensive homology to the NH_2-terminal sequence derived from purified human VLA α_1 (Takada et al., 1987). The sequence matched human α_1 at 12 out of 15 positions but no more than 6 out of 14 when compared with any other α subunit sequence (Fig. 1). The purified protein therefore most likely represents the rat homologue of the human VLA-1 α subunit. Moreover, the human and rat α subunits have similar M_rs of ~200 kD in SDS-PAGE, under reducing and nonreducing conditions (Turner et al., 1989; Kramer and Marks, 1989). In each species this is a relative molecular mass quite distinct from that of other integrin α subunits. Both also exhibit RGD-independent, divalent cation-dependent binding to LN and collagen (Ignatius and Reichardt, 1988; Kramer and Marks, 1989; Hall et al., 1990). Therefore, the purified rat α subunit defined by the 3A3 antibody appears to be the rat homologue to the human VLA-1 α subunit.

cDNA Cloning and Nucleotide Sequence

A 48-base oligonucleotide deduced from the NH_2-terminal sequence of the antibody-purified protein was used to screen a PC12 cell cDNA library. Several positive clones were identified and shown to contain α_1 cDNA inserts by sequencing of PCR products. Two independent clones containing full length inserts in opposite orientations, were sequenced. Translation of the resulting 3,987 bp of nucleotide sequence (Fig. 2) reveals an open reading frame of 3,456 bp encoding a 1,152-amino acid mature protein with a 28-amino acid signal sequence. A perfect match of 24 amino acids of sequence from the NH_2 terminus of the purified protein with sequence deduced from the translation of the cDNA, establishes the authenticity of the clone (*thick underline*, Fig. 2). The NH_2-terminal sequence of the mature protein is preceded by a translation start site that codes for a methionine in the proper context (Kozak, 1987) followed by a 27-amino acid long putative signal peptide. None of the other five upstream ATGs are in the proper context for initiating translation and all are followed by in frame stop codons within 400 bases. The predicted site of signal peptide cleavage fulfills the −1/−3 rule of von Heinje (1986) and agrees with the NH_2-terminal amino acid determined by protein sequencing. The short string of As in the 3′ end of the clone seems unlikely to reflect the actual polyadenylation site. There is no upstream AATAAA or ATTAAA sequences which precede 85% of all polyadenylation sites (Proudfoot and Brownlee, 1974) nor is it likely that the additional 6–7 kb in the mature message (see below) is entirely poly A or in the 5′ untranslated region. Instead some of these As may be derived from the oligo-dT primer used during construction of the cDNA library and are not necessarily all a part of the mature message.

Amino Acid Sequence of the Mature Protein

The mature protein is similar in structure to other integrin α subunits. It contains a large extracellular domain, a single transmembrane domain and a short cytoplasmic tail. The predicted molecular weight for the mature peptide of 1,152 amino acids is 127,752 D. The presence of 24 potential N-glycosylation sites (Asn-Xaa-Ser/Thr), predicted to contribute an average of 2,500 D to the M_r of the core protein (Parham et al., 1977), would yield a predicted M_r for the mature protein on SDS-PAGE of 187 kD. This is similar to the M_r of α_1 observed on SDS-PAGE gels where the mobility is ~185 kD (nonreduced) and 200 kD (reduced) (Turner et al., 1989). Sequence analysis also predicts a single transmembrane domain of 23 residues (*underline*, Fig. 2). This is followed by a short cytoplasmic tail of 15 amino acids including the sequence, GFFKR, common to all vertebrate integrin alpha subunits sequenced so far.

Analysis of human and rat Mac-1 alpha subunits revealed seven repeats of a domain in the most distal NH_2-terminal region (Pytela, 1988). Each repeat is distinguished by borders of conserved sequence along with the conservation of internal glycine residues. As shown in Fig. 3, a similar sevenfold repeat is seen in the sequence of the rat α_1 homologue. Each repeat contains four to five glycines and analysis of the aligned repeats shows that there is 78% conservation of residues appearing in the similar position in three or more of the repeats. Of the 38 conserved residues, 20 are hydrophobic. The 206 residue I-domain (described below) lies outside of these repeats between repeats 2 and 3.

Three domains in repeats 5, 6, and 7 are probable divalent cation or metal binding sites (dashed underline in Fig. 2, solid underline in Figs. 4 and 5). These domains have been described for all other integrin alpha chains sequenced and are somewhat similar to consensus metal-binding domains of other Ca^{2+}- and Mg^{2+}-dependent proteins with the sequence DxD/NxD(G) xxD (Reinach et al., 1986; Vyas et al., 1987).

An odd number of cysteines (23) in the extracellular domain of the mature peptide suggests that one or more cysteines may exist in an unpaired, reduced form, or are paired with other cysteines in adjacent accessory protein(s). Errors due to cDNA synthesis or DNA sequencing are unlikely to have produced this odd number since two unique clones were independently sequenced and yielded the same sequence. An odd number of cysteines has also now been seen in the extracellular domain of the chicken integrin α_{vnr} subunit (Bossy, B., and L. F. Reichardt, personal communication).

Homology of Rat Integrin α_1 to Other Proteins

A search of GenBank showed homology to all of the known

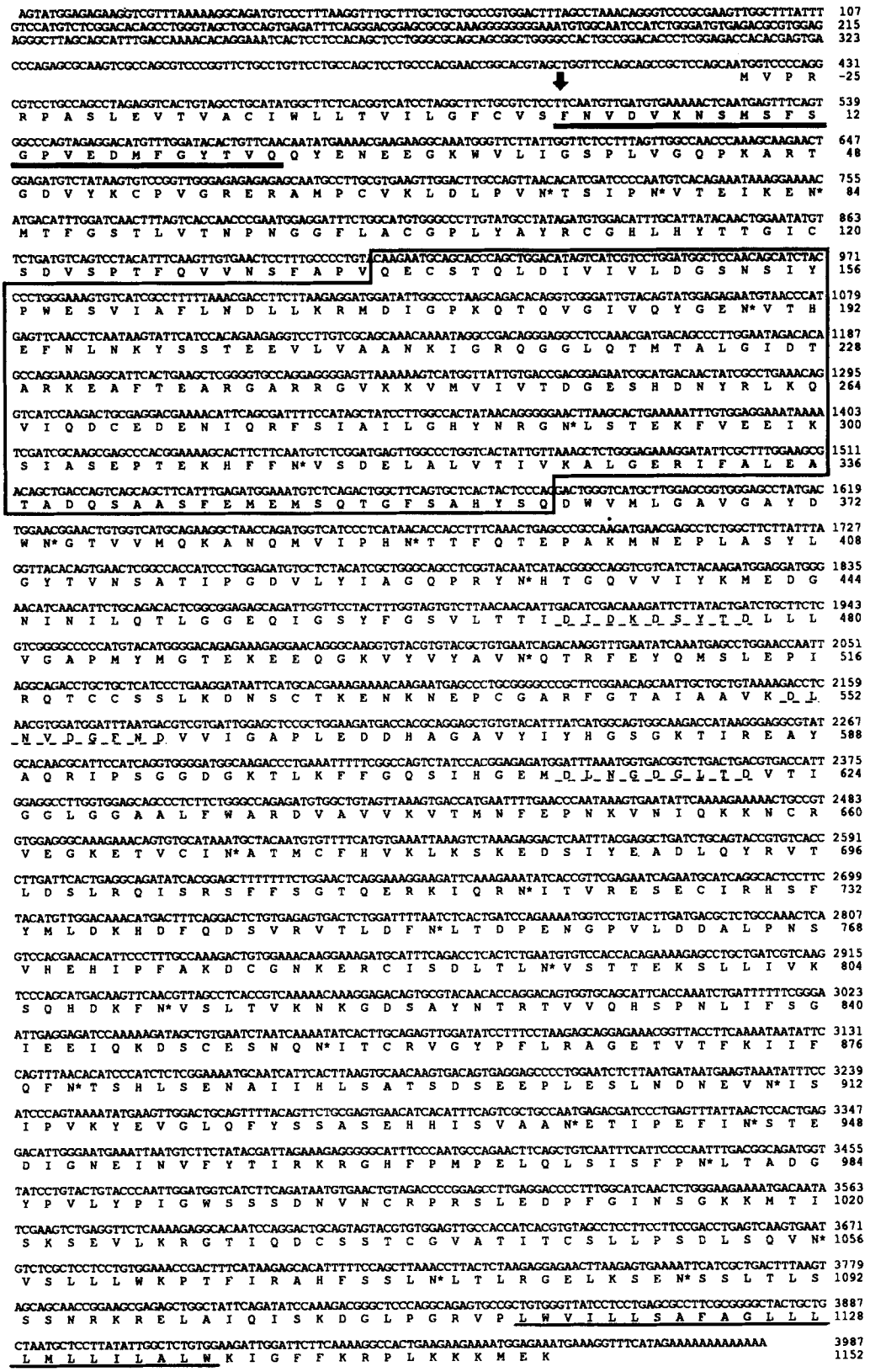

Figure 2. Complete nucleotide sequence of a rat α_1 cDNA clone and its translated amino acid sequence. An arrow shows the start of the mature peptide, which is preceded by a 27-residue-long signal peptide. The NH_2-terminal sequence derived from the purified protein is shown underlined and matches the deduced amino acid sequence. The I domain is contained within the boxed area; probable divalent cation binding sites are indicated by dashed underlines and the transmembrane domain by a solid underline. Asterisks mark possible N-glycosylation sites (NxT/S). See Fig. 6 for a schematic summary of this structure. These sequence data are available from EMBL/GenBank/DDBJ under accession number X52140.

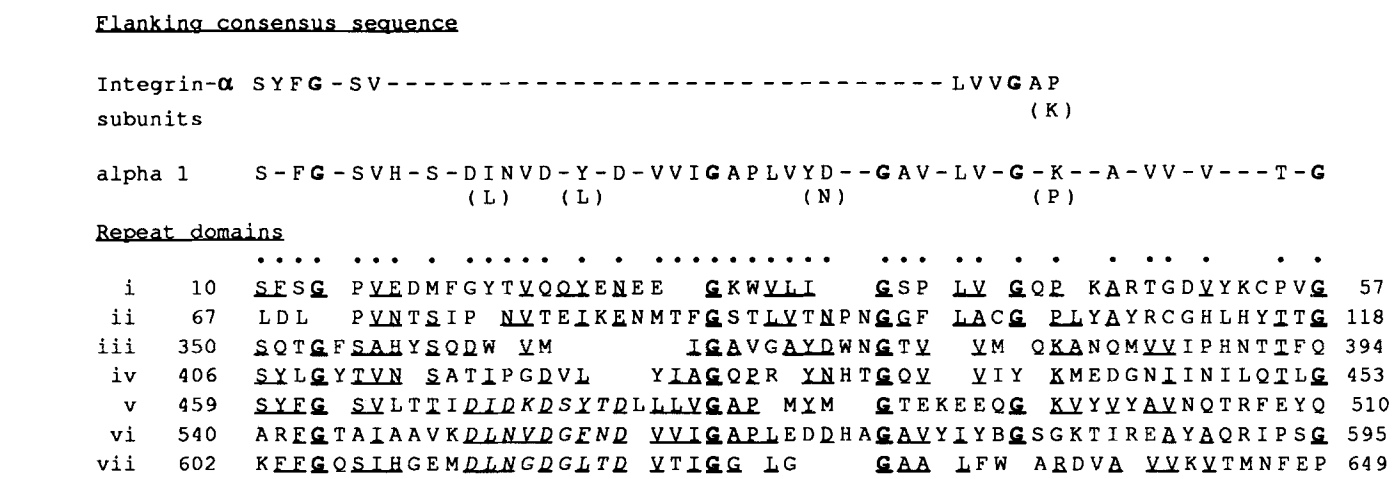

Figure 3. Sequence structure of seven repeat domains in rat α_1. Alignment of these seven regions in the distal portion of rat α_1, with an average length of 49 residues, reveals a conserved, repeated structure. Amino acids identical or conserved in three or more repeats are underlined, and marked with a (•) placed over their position (conservative substitutions are *I, V, L, A*; *E, D, Q, N*; *Y, F, W*; *S, T*; *A, G*; *K, R*). Four to five glycine (*G*) residues (in bold type) are conserved in each repeat. The probable divalent cation binding sites are in italics. Flanking consensus sequences for integrins are according to Larson et al. (1989). These sequence data are available from EMBL/GenBank/DDBJ under accession number X52140.

integrins (GenBank release no. 62 [12/89]). In addition, a subdomain of the sequence showed homology to domains within collagen VI (Chu et al., 1989), von Willebrand factor (Shelton-Inloes, 1988), chicken cartilage matrix protein (Kiss et al., 1989) human complement factors B (Mole et al., 1984) and C2 (Bentley, 1986). The homology to the above matrix proteins is contained within a 206-amino acid "I" domain (for inserted or interactive, Figs. 2 and 4). The presence of this domain has been proposed as a site within both the receptor and these matrix proteins for interacting with collagens or with components of basement membranes (see Pytela et al., 1988).

Comparison of the Rat Integrin α_1 Sequence to Other Integrin Alpha Chains: Alignment with I Domain-containing α Subunits

The deduced amino acid sequence presented here for integrin α_1 is similar to all other known integrin alpha subunits and the relative extent of this homology to the known human sequences are revealed in their alignment scores listed in Table I. The α_1 subunit is most similar to the VLA-2 α subunit (40% identical) with an overall alignment score of 319. The α_1 and α_2 subunits are 52% identical within the first 650 residues (which span the extent of the seven repeats), and are 61% identical within their I-domains. The remaining 490 extracellular residues between the end of the repeats and the transmembrane domain are only 29% identical. Like VLA α_2, the rat integrin α_1 subunit is most homologous to other alpha subunits of integrins that contain "I" domains (LFA-1, Mac-1, and p150). Moreover, α_1 shares other properties with I-domain containing integrin α subunits. α_1 contains only three potential divalent cation binding sites and no obvious disulfide bridged cleavage site corresponding to the known cleavage site around position 850 in cleaved subunits, such as the fibronectin receptor α_5 subunit.

Comparison of α_1 with the four I-domain containing alphas reveals overall structural similarity and regions of strong homology (Fig. 4). Between these five α subunits, 22% of the residues are identical or conservative substitutions (see legend in Fig. 3 for definition of conservative substitutions used here). Most of this conservation is confined to the NH_2-terminal three-fifths of the protein and the transmembrane domain. In the first 650 residues, 28% of the amino acids are identical or conserved, while in the more proximal COOH-terminal 490 residues, excluding the transmembrane domain, this number is only 11%. The 22 residues of the predicted transmembrane region are 70% conserved. The total number of amino acids itself is highly conserved between individual mammalian I-domain alpha subunits, with a variability in lengths of only 15 amino acids from 1,137 to 1,152. Thus, the broad range in relative molecular masses of alpha subunits seen with SDS-PAGE (140 to 200 kD) must primarily reflect differences in glycosylation.

16 out of the 23 cysteines in rat α_1 can be aligned with cysteines in the four other I domain-containing alphas. 10 of the 16 are conserved in non-I domain alpha subunits as well (Fig. 4; residues that are conserved in both sets of alignments are indicated by underlined dots above the sequence). Both the boundaries and extent of the I domains are conserved, with overall conservation of 54 out of the 206 residues or 26%. Within the I domain, three regions, defined by residues 144–156, 239–255, and 331–338 exhibit the highest degree of homology. In the alignment of the I domain in Mac-1 with seven matrix proteins (Pytela, 1988) these same regions were the most highly conserved as well, indicating an important structural or functional role for these subdomains.

After the I domain are the three, conserved divalent cation binding domains. All of the alpha subunit sequences share a short segment, GxQIGSYFGxxL (position 454–465) just NH_2-terminal of the first putative metal binding domain. Between the second and third domains, in a stretch of 53 residues, 53% of the residues are conserved among I domain alphas and more than half of these are conserved among all human and rat alphas.

Between cation binding domains 1 and 2, however, there is a region that is poorly conserved among different α

α1 (1152 amino acids)
α2 (1152 amino acids)
αmac1 (1137 amino acids)
αp150 (1144 amino acids)
α1fa1 (1145 amino acids)

subunits. In rat α_1 this segment is nearly twice as long as the equivalent regions in all other alphas (position 501–539 marked by italics in Figs. 4 and 5). Of particular significance is the presence of four cysteines in the rat α_1 in a portion of this segment that is predicted to be hydrophilic and therefore is likely to be exposed. Because the sequence is found in a region of integrin α subunit proposed to be involved in ligand binding (Smith and Cheresh, 1990), it may well confer a unique ligand-binding function to the α_1 chain.

Alignment with Non–I domain-containing Integrin α Subunits

To compare the structure of α_1 with that of non-I domain containing integrin alpha subunits, the I domain (residues 144–349) was removed from the sequence and then the remainder aligned with α_4, α_5, α_{vnr}, and α_{IIb} (Fig. 5). The general alpha subunit structure is preserved, including the positions of cysteines (14 out of 23) and of three putative metal binding domains. Overall, 177 out of 967 residues (18%) are conserved between α_1 and the four non-I domain alphas. Of the 177 conserved residues, 103 are shared with all alphas. Like the I domain alphas, these are concentrated in specific regions, primarily around the metal binding domains where 107 out of 420 or 25.4% of the residues are shared. Yet a similar divergence in both the extent and content of the sequences is found in the region between metal binding domains 1 and 2, where the nonconserved, 38 residue segment, with four cysteines is found rat α_1 (italics, Fig. 5). In the NH_2-terminal and cytoplasmic portion of the protein, excluding the transmembrane and GFFKR, only 9% of the amino acids are conserved. The transmembrane domain is conserved in 12 out of 23 positions (52%).

Potential Cleavage Sites

Reduction of three integrin alpha subunits, α_5, α_{vnr}, and α_{IIb} releases a small COOH-terminal fragment, normally attached via a disulfide linkage (Argraves et al., 1987; Suzuki et al., 1987; Loftus et al., 1988). This configuration is produced by a dibasic cleavage site (K/R-R-E/D) followed by several hydrophobic residues described for each cleaved alpha around position 850, but missing from I domain-containing alphas. A Lys-Arg pair is seen in this position in both α_1 and α_4 but it is not followed by an acidic E or D residue or any hydrophobic residues. Because neither α_1 or α_4 is cleaved at this site (Ignatius and Reichardt, 1988; Hemler et al., 1987; Turner et al., 1989), this suggests that the additional acidic and hydrophobic residues may be required for cleavage at similar dibasic sites.

There is an additional putative protease cleavage site, unique to rat α_1, at position 1,095 with the sequence NRKRELA, that fulfills the criteria described above. Cleavage at this site would eliminate the covalent association of the two proteolytic fragments, since no cysteines are present in the smaller fragment. The size of the smaller fragment predicted from cleavage at this position (~6,200 D) would make it difficult to detect directly. Published experiments do not address critically the possibility that the integrin α_1 subunit is cleaved at this site. In Fig. 6, a schematic representation of the alpha subunit structure is shown, with the position of this potential site indicated.

RNA Analysis

Poly A+ RNA prepared from PC12 cells was separated on an agarose-formaldehyde gel, transferred to a nitrocellulose filter, and hybridized with a full-length clone encoding rat integrin α_1. A band of ~11 kb was detected (Fig. 7). This indicates that the cDNA coding for the entire protein was derived from a much larger mRNA. It seems likely that much of the mRNA sequence is in 3′ untranslated RNA, but that has not yet been proven.

Discussion

We describe here a cDNA clone of the rat homologue of the human VLA-1 integrin alpha subunit, initially identified on human lymphocytes (Hemler et al., 1987). Identification of the full-length cDNA as coding for the 3A3 antigen is based upon the perfect match of sequence derived from the cDNA with that obtained by microsequencing of the protein isolated with the 3A3 antibody. The preservation of a variety of integrin specific structural domains as reported here further establishes this protein as a member of the integrin family.

Although little is known about the function of human VLA-1 on lymphocytes, a number of results demonstrate that the 3A3 antigen is the rat homologue of VLA-1 and that both α subunits associate with a β_1 subunit to form a receptor for LN and collagen. Out of 105 residues of amino acid sequence derived from peptide fragments of the human VLA-1 alpha subunit, 90% are identical, and 95% are conserved with the rat integrin sequence derived in this study (Crouse, C., and M. E. Hemler, personal communication). Antisera to human VLA α_1 immunoprecipitate a 200 kD/120 kD heterodimer and inhibit the adhesion of human cells to several collagens, intact LN and the E1 fragment of LN (Hall et al., 1990). Neither the human or rat $\alpha_1\beta_1$ receptors interact with the E8 fragments of LN (Hall et al., 1990; Tomaselli K. J., and L. F. Reichardt, personal communication). This LN fragment specificity is different from that of other integrins, such as $\alpha_3\beta_1$, and $\alpha_6\beta_1$, which interact with LN primarily using sites in the E8 or long arm fragments of LN (Gehlsen et al.,

Figure 4. Alignment of rat α_1 with other I-domain containing integrin alpha subunits. The rat α_1 sequence is compared with human sequence for integrins α_2 (Takada and Hemler, 1989), $\alpha_{Mac\text{-}1}$ (Arnaout et al., 1988; Corbi et al., 1988), α_{p150} (Corbi et al., 1987), and $\alpha_{LFA\text{-}1}$ (Larson et al., 1989). The 206-amino acid-long I domain and three divalent cation binding sites are underlined. Identical residues matched in two or more sequences are capitalized, and aligned cysteines are shown in bold type. Residues identical or conserved among all five sequences are designated with a (·) above them and with an underlined dot, (̣), where these residues are also conserved in the alignment of all other integrin alpha subunits (Fig. 5). Conserved residues are as defined in Fig. 3. A 38-residue segment unique to rat α_1 is shown in italics. These sequence data are available from EMBL/GenBank/DDBJ under accession number X52140.

Table I. Summary of Scoring Matrix

		1	2	3	4	5	6	7	8	
β_1	1. α_1	–								
	2. α_2	319	–							
	3. α_4	180	198	–						
	4. α_5	174	187	214	–					
β_2	5. α_{LFA}	192	189	180	150	–				
	6. α_{MAC1}	200	216	180	185	270	–			
	7. α_{p150}	201	211	208	189	277	573	–		
β_3	8. α_{VNR}	164	172	210	442	150	163	175	–	
	9. α_{GPIIB}	168	ND	185	336	ND	ND	ND	342	–

Alignment scores shown: $M - (G \times L)$, where M = no. of identical matches, G = no. of gaps and L = length of gap.

1989; Hall et al., 1990). Thus, the rat α_1 subunit described here shares NH_2-terminal and internal sequence homology, apparent molecular weight, association with the same β_1 subunit, and adhesive specificity with human VLA-1.

Conservation of Structural Domains

Alignment of the rat α_1 amino acid sequence with other members of the integrin family suggest two classes of integrin α subunits appear to have evolved; subunits containing an I domain and subunits without an I domain. Subunits containing an I domain are not cleaved into disulfide-linked heavy and light chains and have only three metal binding domains. Most non–I domain-containing alpha subunits have four metal binding domains and are proteolytically cleaved at a conserved site, which is flanked by cysteines that covalently bind the large and small fragments together. The noncleaved VLA-α_4 is an exception to these tentative rules, because it has three metal binding domains, no I domain, and a potential cleavage site that is quite distinct from the other cleaved alpha subunits (Takada and Hemler, 1989). The rat α_1 protein sequence includes an I domain and three metal binding domains and does not appear to be proteolytically cleaved at a site that is bridged by a covalent disulfide linkage. It is, therefore, a member of the I domain family of integrin receptors along with VLA-2, Mac 1, LFA-1, and p150.

As described above, the rat α_1 sequence is most homologous to VLA α_2. Linkage analysis based on alignment scores predicts that VLA α_2 shares the closest ancestral precursor with the three alpha subunits in the β_2 family (Takada and Hemler, 1989; Takada et al., 1989). By extension, α_1, sharing the highest degree of homology with α_2, is likely to have evolved most recently from a progenitor shared with α_2 and more distantly with a progenitor shared with other I domain-containing integrins. This ancestral ordering does not merely reflect the presence of the I domain, because the same ranking of alignment scores is generated using the integrin sequence with I domains removed (Table I).

The "I" domain (for inserted or interactive) is a 206-amino acid insert. It shows homology to domains in several matrix proteins including collagen VI (Chu et al., 1989), von Willebrand factor (Shelton-Inloes, 1986), chicken cartilage matrix protein (Kiss et al., 1989) and complement factors B and C2 (Mole et al., 1984; Bentley, 1986). Several of these proteins have been shown to interact with collagen or other matrix proteins in the assembly of basement membranes, possibly through this conserved region (see Chu et al., 1989). It has been suggested that the I domain may be the domain in integrin alpha subunits that interacts with similar matrix proteins (Pytela, 1988). Consistent with this hypothesis, both integrin $\alpha_1\beta_1$ and $\alpha_2\beta_1$ heterodimers have been shown to bind collagen in a divalent cation-dependent manner (Santoro et al., 1988; Staatz et al., 1989; Kramer and Marks, 1989; L. M. Goetzl, M. Ignatius, and L. F. Reichardt, personal communication). However, the absence of evidence documenting collagen binding by the I domain-containing receptors in the β_2 family suggests that additional structures may be required for this specificity or that not all I domains mediate interactions with collagen.

Divalent cations are important for integrin function, through either stabilization of receptor ligand interactions which require millimolar concentrations (Marlin and Springer, 1987; Cheresh et al., 1987) or $\alpha\beta$ subunit association which requires at least micromolar concentrations of cation (Fitzgerald and Phillips, 1985; Holzman et al., 1988). All alpha subunits characterized to date, including the rat α_1 described here have three or four metal binding domains. Binding of cations in this region may stabilize tertiary structure in these receptors necessary for ligand binding since all integrins described to date exhibit divalent cation dependent ligand binding (Marguerie et al., 1980; Gailit and Ruoslahti, 1988; Ignatius and Reichardt, 1988; Gehlsen et al., 1988; Kramer and Marks, 1989). Interestingly, the rat $\alpha_1\beta_1$ heterodimer exhibits a strong dependence on Mg^{2+} (Turner et al., 1989), and all other I domain-containing integrins are Mg^{2+} dependent (Santoro, 1986; Marlin and Springer, 1987; Staatz et al., 1989).

Possible Ligand Binding Site within Alpha-1

The rat α_1 sequence diverges from the other integrin alpha subunits in a region between the first and second metal binding domains. This segment is 38 residues long, contains four nonconserved cysteines, and is flanked by regions of extensive homology. Other alphas show weak homology in this region and include only about half the number of residues. The location of this insert in a region that may have a conserved function in ligand binding by integrin receptors (Cheresh and Smith, 1990) suggests that it may impart a unique function, possibly in ligand recognition, to this receptor. The presence of four nonconserved cysteine residues also indicates a significant structural divergence.

An alternatively spliced form of PS2, an integrin homologue in *Drosophila melanogaster*, contains a 25-amino acid exon corresponding to a region ~50 amino acids NH_2-

Figure 5. Alignment of rat α_1 with other integrin alpha subunits lacking an I domain. The rat α_1 sequence is compared with the human sequence for integrin alpha subunits α_4 (Takada et al., 1989), α_5 (Argraves et al., 1987; Fitzgerald et al., 1987), α_{vnr} (Suzuki et al., 1987), and α_{IIb} (Poncz et al., 1987). The I domain, from positions 144 to 349 is deleted from the rat α_1 sequence. All other symbols are as described in the legend for Fig. 4. These sequence data are available from EMBL/GenBank/DDBJ under accession number X52140.

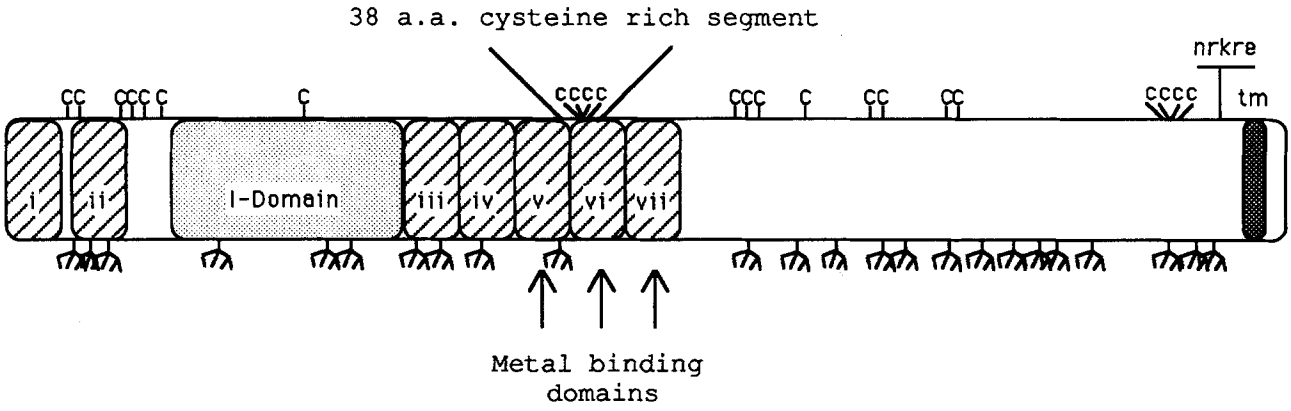

Figure 6. Schematic representation of the structure of rat α_1. The position of all 23 cysteines are indicated by Cs and the position of 24 probable N-glycosylation sites is indicated by the branched stick figures. Individual domains are drawn to scale on the horizontal axis. The position of a potential protease site near the transmembrane domain is shown with its sequence and include the seven repeated domains designated by diagonal lines.

terminal to the first metal binding domain in α_5, α_{vnr}, and α_{gpIIb} or 100 amino acids NH_2-terminal to the first conserved metal binding domain in other alpha subunits (Brown et al., 1989). Designated variable region A, it is flanked by domains highly conserved in all integrin alphas and is proposed as playing a role in determining ligand specificities (Brown et al., 1989). The 38-amino acid insert in rat α_1, located another 100 amino COOH-terminal to the end of this variable region could represent an α_1-specific exon that serves a similar function in designating ligand specificity.

The integrin β_1 family includes at least four α subunits: α_1, α_2, α_3, and α_6, which form heterodimers that interact with LN (Sonnenberg et al., 1988; Ignatius and Reichardt, 1988; Gehlsen et al., 1988; Languino et al., 1989; Elices and Hemler, 1989; Hall et al., 1990). Recent studies indicate that for at least VLAs 1, 3, and 6 the regions of LN bound by these receptors are different. Elastase digestion of LN generates an E1 fragment, to which human and rat $\alpha_1\beta_1$ can bind, whereas another distinct fragment E8 is bound by human $\alpha_6\beta_1$ (Hall et al., 1990) and rat $\alpha_3\beta_1$ heterodimers (Gehlsen et al., 1989). The regions of LN bound by $\alpha_2\beta_1$, the integrin alpha subunit most homologous to α_1, are not known. At this point sequence information is only available for two of the integrins able to bind LN (α_1 and α_2). When the LN fragment preference for $\alpha_2\beta_1$ are determined along with the primary structure of α_3 and α_6, structure-function correlations may be apparent that establish the specific ligand binding properties for these receptors.

A confounding problem in many biological assays of integrin function is the coexpression on individual cells of multiple integrins, some of which bind the same ligand, e.g., LN. A second problem, only recently appreciated, is the existence of multiple isoforms of LN derived in part from new genes (Hunter et al., 1989). It is possible that receptors within the β_1 family of LN receptors can distinguish between these isoforms. The molecular cloning of the rat homologue of the human VLA-1 alpha subunit will now allow experiments, including the production of stable transfectants in cells devoid of other receptors that will generate a more precise designation of the ligand-binding specificities of this receptor.

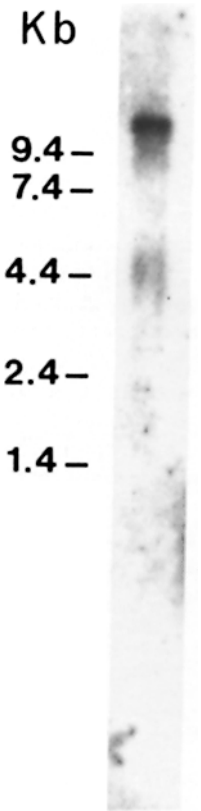

Figure 7. mRNA blot analysis of PC12 cells using the rat α_1 cDNA as probe. For Northern blots (*A*) poly A+ RNA (5 μg) isolated from PC12 cells was probed with the full-length, 3.9-kb rat α_1 cDNA. A major band is apparent at ~11 kb and a fainter broader band at 4.4 kb that is possibly a degradation product of the larger mRNA. Essentially identical results were obtained in two other experiments.

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