The Primary Structure of the VLA-2/Collagen Receptor α^2 Subunit (Platelet GPIa): Homology to Other Integrins and the Presence of a Possible Collagen-binding Domain

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Abstract. VLA-2 (also called gpIa/IIa on platelets) is a collagen receptor with a unique α subunit and a β subunit common to other adhesion receptors in the VLA/integrin family. Multiple cDNA clones for the human VLA-2 α^2 subunit have been selected from a λgtll library by specific antibody screening. The 5,374-bp nucleotide sequence encoded for 1,181 amino acids, including a signal peptide of 29 amino acids followed by a long extracellular domain (1,103 amino acids), a transmembrane domain, and a short cytoplasmic segment (22 amino acids). Direct sequencing of purified α^2 protein confirmed the identity of the 15 NH_2-terminal amino acids. Overall, the α^2 amino acid sequence was 18–25% similar to the sequences known for other integrin α subunits. In particular, the α^2 sequence matched other integrin α chains in (*a*) the positions of 17 of its 20 cysteine residues; (*b*) the presence of three metal-binding domains of the general structure DXDXDGXXD; and (*c*) the transmembrane domain sequence. In addition, the α^2 sequence has a 191-amino acid insert (called the I-domain), previously found only in leukocyte integrins of the β_2 integrin family. The α^2 I-domain was 23–41% similar to domains in cartilage matrix protein and von Willebrand factor, which are perhaps associated with collagen binding. The NH_2-terminal sequence reported here for α^2 does not match the previously reported α^2 NH_2-terminal sequence (Takada, Y., J. L. Strominger, and M. E. Hemler. 1987. *Proc. Natl. Acad. Sci. USA.* 84:3239–3243). Resolution of this discrepancy suggests that there may be another VLA heterodimer that resembles VLA-2 in size but has a different amino acid sequence.

VLA-2 is an α/β-subunit cell surface heterodimer strongly implicated as receptor for collagen because (*a*) VLA-2 has been shown to be identical (61) to a 150,000/110,000-M_r structure recognized by the mAb PlH5, which specifically blocks human fibrosarcoma cell (66) and platelet (31) attachment to collagen; (*b*) patients deficient in platelet protein Ia (α subunit of VLA-2) also lacked responsiveness to collagen (27, 41); and (*c*) the mAb 12F1 was used to identify VLA-2 as a 160,000/130,000-M_r (nonreduced) platelet protein complex that mediates Mg^{2+}-dependent adhesion to collagen (51, 52). Also in this regard, antigens that strongly resemble VLA-2 in size have been implicated in hepatocyte cell attachment to type I collagen (14).

The cell surface heterodimer VLA-2 was initially characterized as a "very late antigen" appearing on activated T cells (19, 20). Later the mAb 12F1 was used to identify VLA-2 on platelets and on many other cell types, including most rapidly growing, adherent cell lines (43). Major portions of the platelet gpIa and IIa are probably the same as the VLA-2 α and β subunits, respectively (31, 44). Thus the new platelet-specific alloantigens Br^a and Br^b, which reside on the platelet Ia–IIa complex (53), may be variable epitopes on VLA-2.

In other studies, an antibody (called 5E8) that recognizes VLA-2 (Hemler, M., and C. Crouse, unpublished results) has been described on most primary human lung tumors (68), and ^{125}I-coupled 5E8 antibody has been used to inhibit the growth of lung tumor cell lines in vitro (57).

The expression of VLA-2 can be up regulated on lymphocytes in response to mitogen or antigen stimulation (17) and on fibroblasts in response to serum (10). Conversely, VLA-2 expression slowly diminished when those stimuli were withdrawn and/or cells became quiescent (10, 17). Also, VLA-2 expression can be up regulated by TGFβ (15).

Like other cell surface receptors for extracellular matrix components, VLA-2 belongs to the integrin superfamily (26, 49). Three subfamilies of that superfamily that are defined for humans are (*a*) the six (or more) VLA proteins (16, 23); (*b*) the leukocyte adhesion molecules, LFA-1, Mac-1, and p150,95 (56); and (*c*) the cytoadhesins, which include the vitronectin receptor and platelet gpIIb/IIIa (13). Within each

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family, the member heterodimers are each composed of a unique α subunit associated with a common β subunit. Analyses of human cDNA clones for the β subunits of each subfamily (called β_1, β_2, and β_3, respectively) have revealed that they are 44–47% similar (2, 12, 28, 33, 62), suggesting a common evolutionary origin. The evolutionary conservation of β_1 is emphasized by the homology (82–86%) maintained between β_1s from widely diverging species, such as human, chicken, and frog (9), and 45% homology between human β_1 and a β_1-like structure from *Drosophila* (36). Also, complete sequencing of several integrin α subunits—including those from a fibronectin receptor (α^5) (2, 11), the vitronectin receptor (58), platelet gpIIb/IIIa (11, 45), Mac-1 (3, 8, 46), p150,95 (7), and the *Drosophila* PS2 antigen (6)—have revealed 20–60% similarity between any pair. Some of these integrin structures are receptors for ligands containing the amino acid signal sequence Arg-Gly-Asp (RGD) or a closely related sequence (49). Although NH_2-terminal amino acid sequencing has suggested ~40% shared residues among six different VLA α subunits (24, 60), complete sequence information is available for only one VLA α subunit (α^5) (2, 11). Thus, to further establish patterns of similarity and differences among VLA/integrin sequences and to gain basic information for future studies of structure, function, and regulation, the complete sequence of the VLA α^2 subunit was obtained. Also, because the α^2-subunit NH_2-terminal sequence predicted from cDNA did not match the previously published "α^2" NH_2-terminal sequence, experiments were carried out to resolve this discrepancy.

Materials and Methods

Purification of VLA-2 from Platelets and NH_2-terminal Sequencing

VLA-2 protein was purified from outdated platelets (from the Dana-Farber Cancer Institute [Boston, MA] blood bank or from the American Red Cross [Dedham, MA]) by lectin-Sepharose and then by 12F1 or A-1A5 immunoaffinity chromatography (60). The anti-α^2 mAb 12F1 (43) and the anti-β_1 mAb A-1A5 (18) were obtained as described. For NH_2-terminal amino acid microsequencing of the α^2 subunit, the α^2 subunit was first separated from the β subunit by SDS-gel electrophoresis, transferred to a polyvinylidene difluoride membrane (Immobilon; Millipore Continental Water Systems, Bedford, MA), stained with Coomassie blue, and destained as described (39). Excised membrane pieces containing α^2 material were then sequenced using a gas-phase sequenator (470A; Applied Biosystem, Inc., Foster City, CA) equipped with a phenylhydantoin amino acid analyzer (120A; Harvard Microsequencing Facility, Cambridge, MA).

Preparation of α^2-Subunit-specific Antiserum

Subunits migrating in the α^2 position (~50 μg from A-1A5-Sepharose or 12F1-Sepharose) were reduced and separated using preparative 5% SDS-PAGE, stained with Coomasie blue, and destained in 7% acetic acid and 30% methanol. The band corresponding to α^2 peptide was cut out and electroeluted into Tris/glycine/SDS buffer using an Elutrap (Schleicher & Schuell, Inc., Keene, NH). The eluted peptide was used for rabbit immunization as previously described (23). Also, α^2 protein purified from 12F1-Sepharose was used for immobilization onto CNBr-Sepharose (Pharmacia Fine Chemicals, Piscataway, NJ) according to the manufacturer's instructions, except that 0.1% of SDS was included in the coupling buffer. Antibodies from rabbit anti-α^2-subunit antiserum were then purified using the α^2-Sepharose affinity column. Briefly, the antiserum was passed through the column several times and the column was washed successively with 3 column volumes each of PBST (0.14 M NaCl, 10 mM sodium phosphate, 0.2% Tween 20), 0.2 M KSCN, and then PBST again. For elution of α^2-subunit-specific IgG, 3 M KSCN was added, and the eluate was desalted by Sephadex G-25 chromatography into PBST in the presence of 0.1% hemoglobin as carrier. Immunopurified IgG was further incubated with denatured *Escherichia coli* protein coupled to Sepharose, and with glycoproteins from Molt-4 cells coupled to Sepharose to remove additional nonspecific reactivity. The Molt-4 leukemic T cell line does not usually express VLA-2 (22).

Production of Antibodies to an α^2 COOH-terminal Synthetic Peptide

A 22-mer consisting of the COOH-terminal 21 amino acids predicted from the α^2 sequence, with an added cysteine at the NH_2-terminal end of the peptide, was synthesized by Multiple Peptide Systems (San Diego, CA). This peptide was coupled to carrier protein (keyhole limpet hemocyanin) using m-maleimidobenzoyl-*N*-hydroxysulfosuccinimide ester (Pierce Chemical Co., Rockford, IL) as previously described (29), and then rabbit antibodies were generated using standard techniques (38).

Isolation of the Gene Encoding the α^2 Subunit

A phage λgt11 expression cDNA library of human lung fibroblast IMR-90 (Clontech Laboratories, Inc., Palo Alto, CA) was screened by using affinity-purified rabbit antibodies against α^2 subunit according to Young and Davis (67), except that alkaline phosphatase-conjugated anti-rabbit IgG was used as a second antibody. Alkaline phosphatase activity was detected by using an immunoscreening system (ProtoBlot; Promega Biotec, Madison, WI) with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl phosphate as substrates. Positive clones from the screening were plaque purified, and then phage DNAs were purified by the plate lysate method (37). After the insert was excised from the phage by Eco RI restriction enzyme digestion, the inserts were separated on agarose gels, electroeluted, and subcloned into pBluescript plasmid (Stratagene, La Jolla, CA). A cDNA library made in λgt11 from endothelial cells was the kind gift from Dr. Tucker Collins (Brigham and Women's Hospital, Boston, MA).

DNA Sequencing

The DNA sequences were determined by the dideoxynucleotide chain-termination method of Sanger et al. (50) using adenosine ^{35}S-5'-[α-thio]triphosphate. To facilitate complete sequencing of both cDNA strands, a series of overlapping deletion clones were made in both directions as described (25) by using the Erase-a-base system (Promega Biotec).

Other Techniques

Southern blot analysis was carried out as described (37). For Northern blot analysis, 5 μg of each RNA sample was electrophoretically separated on 1% agarose gels in buffer containing formaldehyde, transferred to nylon membrane (Nytran; Schleicher & Schuell) (37), and then probed with a nick-translated 5.4-kb α^2-subunit cDNA (in pBluescript) using standard hybridization techniques (37). Western blotting (63) and immunoprecipitation (22) were performed as previously described.

Results and Discussion

Cloning of cDNA with α^2-Subunit-specific Antibody as Probe

Affinity-purified rabbit anti-α^2 antiserum was tested by immunoblotting of whole platelet lysate and found to bind specifically to platelet VLA α^2 subunit (Fig. 1), thus affirming the suitability of the serum for library screening. The affinity-purified antibody was then used for immunoselection of clones from a phage λgt11 expression cDNA library made from human lung fibroblast (IMR-90) since fibroblasts were known to be good source for α^2 protein (21). After subcloning into plasmid, a representative positive clone (clone 2.38, ~1.2 kb) was directly sequenced and found to have partial homology to known sequences of other α subunits in the integrin superfamily. Subsequently, clone 2.38 was used to probe a human endothelial cell λgt11 library (another good source for α^2 expression) since that library had longer inserts. Thus, another clone was selected (clone 2.72, ~5.4 kb), completely sequenced, and found to include all of the coding region and

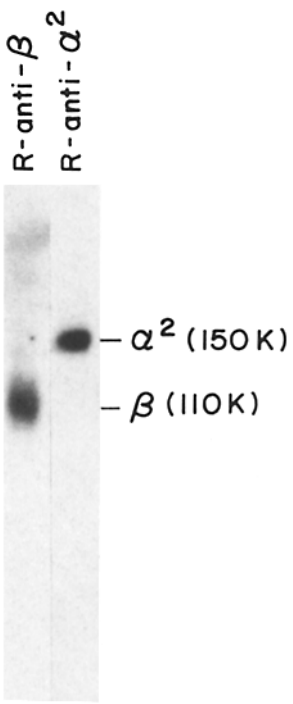

Figure 1. Immunoblotting analysis of rabbit antibody after enrichment for α^2 specificity. After SDS-PAGE separation of purified platelet VLA proteins, the proteins were blotted onto nitrocellulose and then incubated with rabbit anti-β_1 (lane *a*) or anti-α^2 purified (see Materials and Methods) rabbit antiserum (lane *b*). Bound primary antibody was located using ^{125}I-labeled goat anti-rabbit second antibody.

some of the 3′ and 5′ untranslated regions (Fig. 2). Notably, the sequence of clone 2.38 (from a fibroblast library) was identical to the corresponding region in clone 2.72 (from an endothelial cell library).

Comparison of Clone 2.72 cDNA Sequence with α^2-Subunit NH_2-terminal Protein Sequence

Surprisingly, the previously published α^2 NH_2-terminal sequence FNLDTEXDNVFRGP (Fig. 3 *A*) only partly resembled the α^2 NH_2-terminal sequence YNVGLPEAKIFSGPS encoded by cDNA (Fig. 3 *C*), with homology in only 5 of the 14 positions. To resolve this discrepancy, additional NH_2-terminal amino acid sequencing was carried out. Using mAb 12F1–Sepharose, VLA-2 material was purified either from platelets or placenta. After preparative SDS-PAGE, highly purified α^2 subunit from either source yielded an NH_2-terminal amino acid sequence of YNVGLPEAKIFSGPS (Fig. 3 *D*), identical to the sequence encoded from cDNA (Fig. 3 *C*). In another experiment, VLA proteins were purified from 25 g of platelets using the anti-β mAb A-1A5, and the resulting mixture of subunits was then separated by reducing SDS-PAGE so that α^5 and α^6 subunits would migrate at 130,000 M_r, away from the 160,000-M_r α^2 subunit (23). Upon NH_2-terminal amino acid sequencing of the purified β_1-associated 160,000-M_r α^2-like protein from the reduced gel, a mixture of the YNVGLPEAKIFS and FNLTDXEDNVFR sequences was present, with approximately equivalent molar ratios (Fig. 3 *B*). Thus, it appears that the latter sequence may belong to a protein of 160,000 M_r that is distinct from α^2, though it closely resembles α^2 in size. At present, nothing else is known about this potentially new subunit, other than it appears to coprecipitate with β_1 (when using the anti-β_1 mAb A-1A5). Because the mAb 12F1 (originally used to define VLA-2 [43]) was used to isolate the protein with the YNVGLPEAKIFS... sequence, that protein merits the designation as the authentic α^2. It remains to be seen if the recently described multiple forms of platelet gpIa (5) bear any relation to the two distinct NH_2-terminal sequences described here.

Northern Blotting Analysis

The distribution of mRNA for the α^2 subunit was studied by Northern blotting with cDNA clone 2.72 as the probe. A single band at ~8 kb was detected in fibroblast RNA, whereas little or no signal at the same position was obtained in HSB or Molt-4 T cell lines (Fig. 4). These results are consistent with the known cell surface expression of VLA-2 on these cells (21, 22). Hybridization of the same blot with a cDNA probe for the human actin gene gave comparable signals in all lanes. Since the RNA size (8 kb) was somewhat larger than the cDNA clone (5.4 kb) and since no poly A tail is present (Fig. 2), it is assumed that the 3′ untranslated region (and perhaps also the 5′ end) is incomplete.

Authenticity of the α^2 Clone

Despite the unexpected NH_2-terminal sequence results, the α^2 clone obtained is authentic because (*a*) the cDNA was selected using antibodies specific for the α^2 subunit; (*b*) the NH_2 terminus of the predicted sequence was identical to that directly determined from purified α^2-subunit protein; (*c*) the mRNA distribution agrees with that of the mature protein; (*d*) the size of the predicted protein closely agrees with the experimental value (see below); (*e*) the predicted sequence has several features characteristic of other integrin α subunits (see below); and (*f*) native α^2 protein could be readily immunoprecipitated by antisera prepared against a synthetic COOH-terminal peptide including 21 amino acids deduced from the α^2 cDNA sequence (Fig. 5).

As shown in Fig. 5 *A*, rabbit serum directed against an α^2 COOH-terminal synthetic peptide (predicted from cDNA sequence) recognized an $\alpha\beta$ complex (lane *c*) from the colon carcinoma cell line CCL-228 that closely resembled VLA-2 (lane *b*). Prior removal of all material seen by the rabbit serum (lane *f*) resulted in the simultaneous removal of VLA-2 (lane *e*), but only partially diminished total VLA as seen by mAb A-1A5 (cf. lanes *a* and *d*). Also, anti-α^2 COOH-terminal peptide sera specifically immunoblotted α^2 purified from platelets using either the mAb A-1A5 (Fig. 5 *B*, lane *a*) or 12F1 (lane *b*). In comparison, serum from a rabbit immunized with isolated α^2 protein (before anti-α^2 antibodies were purified) recognized mostly α^2 (but also β_1) in preparations obtained from platelets using A-1A5 (lane *c*) or 12F1 (lane *d*), whereas a negative control serum did not blot any detectable purified platelet protein (lanes *e* and *f*).

Amino Acid Sequence of the α^2 Subunit

Translation of the cDNA sequence of clone 2.72 (Fig. 2) yielded coding sequences of 3,543 bp (encoding 1,181 amino acids) between the 3′ and 5′ untranslated portions of ~50 bp and 1,800 bp, respectively. Preceding the NH_2-terminal sequence of the mature protein (YNVGLPE...) is a translational start site (30) that codes for a methionine followed by 29 amino acids, fulfilling the requirements for a signal peptide (64). Thus, the open reading frame shown in Fig. 2 encodes a mature protein of 1,152 amino acids predicted to be

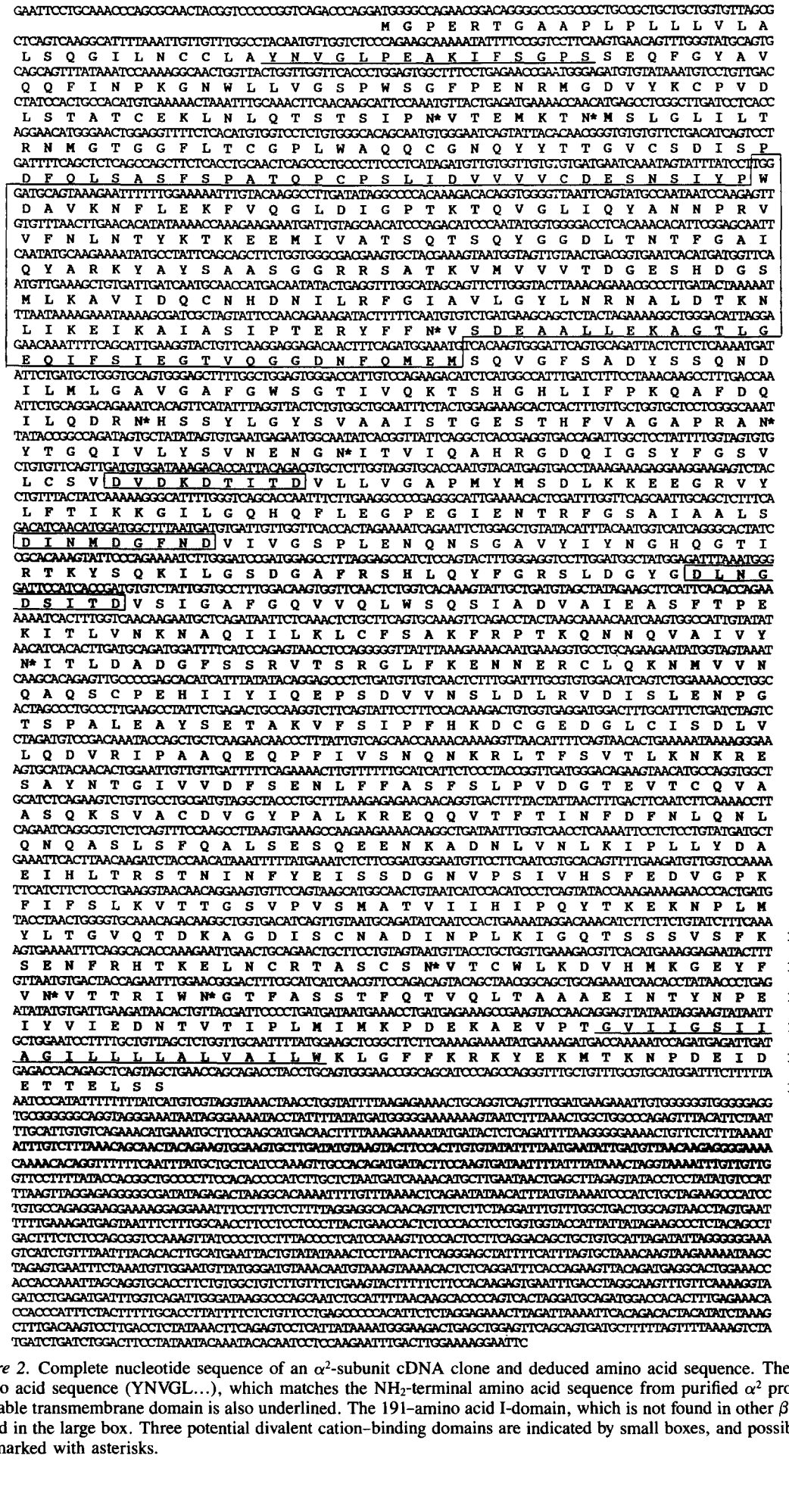

Figure 2. Complete nucleotide sequence of an α^2-subunit cDNA clone and deduced amino acid sequence. The deduced NH_2-terminal amino acid sequence (YNVGL...), which matches the NH_2-terminal amino acid sequence from purified α^2 protein, is underlined; the probable transmembrane domain is also underlined. The 191-amino acid I-domain, which is not found in other β_1 or β_3 integrins, is contained in the large box. Three potential divalent cation-binding domains are indicated by small boxes, and possible *N*-glycosylation sites are marked with asterisks.

	Residue No.:	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
A	α^2 (published as α^2)	F	N	L	D	T	X	E	D	N	V	F	R	G	P	-
B	$\alpha^2 + \alpha^?$	F/Y	N	L/V	D/G	L	P	E	D/A	N/K	I/V	F	-/S	-	-	-
C	α^2 (cDNA)	Y	N	V	G	L	P	E	A	K	I	F	S	G	P	S
D	α^2 (protein)	Y	N	V	G	L	P	E	A	K	I	F	S	G	P	S

Figure 3. Comparison of VLA α^2 and other NH_2-terminal sequences. (*A*) The NH_2-terminal sequence of platelet material previously purified using A-1A5–Sepharose and erroneously published as the α^2 sequence (60); (*B*) the NH_2-terminal sequence from another preparation of platelet material comigrating with α^2 on SDS-PAGE after A-1A5–Sepharose purification of VLA proteins; (*C*) the α^2 NH_2-terminal sequence deduced from cDNA; and (*D*) the α^2 NH_2-terminal sequence obtained after purification of VLA α^2 (from placenta or platelets) using the mAb 12F1 (which is specific for α^2).

126,000 M_r. The addition of 10 potential *N*-glycosylation sequences (Asn-Xaa-Ser/Thr, average 2,500 M_r) to the core protein would result in an estimated molecular mass of 151,000 D. That value is close to the 155,000–160,000 M_r estimated from α^2-subunit migration on SDS-polyacrylamide gels.

Analysis of the NH_2-terminal portion of the sequence revealed the presence of seven homologous repeating domains (Fig. 6). These domains contain 28–41 amino acids and are 20–30% similar to each other. Also, these domains are spaced 23–32 amino acids apart, except that there is a large additionally inserted "I-domain" of 191 amino acids (see below) between repeating domains II and III.

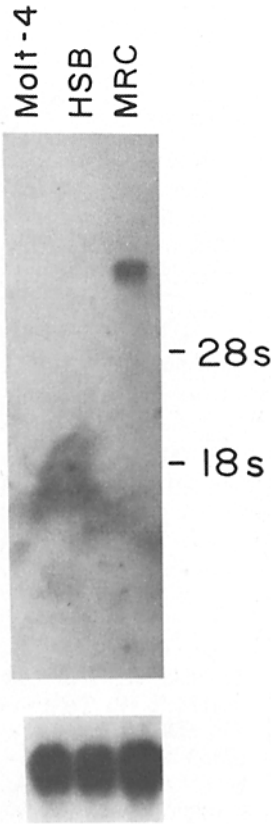

Figure 4. RNA hybridization analysis. Total RNA (5 μg/lane) from the leukemic cell lines Molt-4 and HSB and from the fibroblast cell line MRC was electrophoretically separated on 1% agarose-formaldehyde gels, transferred to nitrocellulose filters, and probed with full-length α^2 cDNA. In a control experiment (*lower panel*), the same RNA samples were probed with actin cDNA.

The repeated domains V, VI, and VII each contain sequences of Dx(D/N)xD(G)xxD (Fig. 2, *small boxes*, and Fig. 6, *underline*) that are somewhat similar to the EF-hand consensus metal-binding domains of a number of calcium- and magnesium-binding proteins, including calmodulin, troponin C, parvalbumin (59), thrombospondin (34), myosin light chain (47), and galactose-binding protein (65). These potential divalent cation-binding sites in α^2 are located in a region (between amino acids 470 and 627) devoid of cysteine residues and *N*-glycosylation sites. The presence of divalent cation-binding sites in the α^2 subunit is consistent with divalent cation requirements for the function of VLA-2 as a human collagen receptor (51, 52).

Comparison of α^2-Subunit Sequence with Other Integrin α Chains

The alignment of the α^2-subunit sequence with the α-chain sequences of human fibronectin receptor (VLA-5), vitronectin receptor, gpIIb/IIIa, Mac-1, and p150,95 shows that several structural characteristics are shared (Fig. 7). For example, of the 20 α^2-cysteine residues, 17 are conserved in at least three of the other sequences and 14 are conserved in all six α-subunit sequences. Also, there is 32–45% conservation in the 22-amino acid transmembrane region and 100% conservation of the GFFKR sequence on the cytoplasmic side of transmembrane domain. Additional striking similarities are evident in the region of the α^2 homologous repeats in the NH_2-terminal half of the molecule. Like α^2, each of the other integrin α subunits has also been noted to have seven homologous repeats, with three or four potential divalent cation sites within repeats IV-VII (2, 3, 7, 8, 11, 45, 46, 58). In these repeat regions, the average similarity between α^2 and the other α subunits ranged from 30–38% (repeats I, II, III, and VII) to 46–52% (repeats IV, V, and VI). The overall similarity between α^2 and the other integrin α subunits is 18–25%, or 22–24% if the I-domain (see below) is excluded. This is in contrast to the higher degree of identity (~45%) between different human integrin β chains (2, 12, 28, 33, 62).

Comparison of the α^2-Subunit I-Domain with Similar Domains in Other Proteins

In studies of Mac-1 (3, 8, 46), p150,95 (7), and LFA-1 (32), an inserted domain of ~200 amino acids was found that is not present in the α^5, α^V, or IIb chains. In those studies, the inserted I-domain (32) (formerly called the L-domain) was noted to resemble the von Willebrand factor (vWF)[1] A1, A2, and A3 domains, cartilage matrix protein (CMP)-1 and CMP-2 domains, and domains in complement factors B and C2. Now an I-domain has also been discovered in the α^2 sequence. The sequence of the α^2 I-domain is aligned with other related domains as shown in Fig. 8, and percent similarity calculations from those data were used in the construction of the linkage tree shown in Fig. 9. The α^2 I-domain sequence (allowing for conservative amino acid substitutions) most resembled I-domains from p150 (48%) and Mac-1 (45%) and also resembled the CMP-1 (46%) and CMP-2 (44%) domains. Less similarity was seen with vWF domains

1. *Abbreviations used in this paper*: CMP, cartilage matrix protein; vWF, von Willebrand factor.

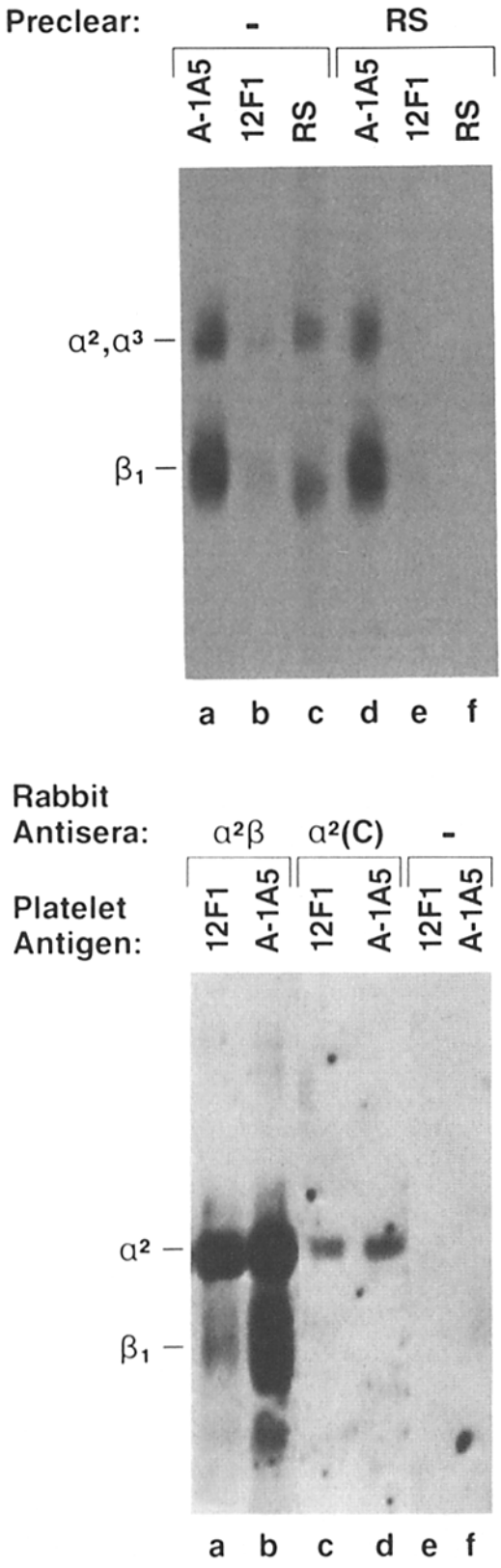

Figure 5. Recognition of α^2 protein by rabbit serum against α^2 COOH-terminal synthetic peptide. (*A*) Immunoprecipitation of VLA proteins from ^{125}I-radiolabeled CCL-228 cells (a colon carcinoma line) was carried out using the mAb A-1A5 (lanes *a* and *d*), 12F1 (lanes *b* and *e*), and rabbit anti-α^2 COOH-terminal peptide (*RS*, lanes *c* and *f*) either with (lanes *d–f*) or without (lanes *a–c*) prior immunodepletion of all rabbit serum-reactive material. (*B*) The mAbs 12F1 and A-1A5 were used to purify either VLA-2 or total VLA protein from platelets (see Materials and Methods). Then aliquots of purified proteins were separated by SDS-PAGE, blotted onto nitrocellulose, and probed as described (63) using rabbit anti-intact α^2 plus β_1 (lanes *a* and *b*), anti-COOH-terminal synthetic peptide (lanes *c* and *d*), or control rabbit serum (lanes *e* and *f*).

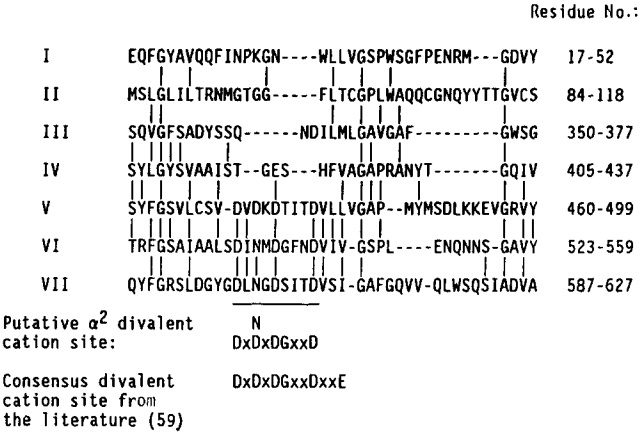

Figure 6. Alignment of seven homologous repeated domains in the α^2 subunit. Residues that are the same in adjacent sequences (including conservative subsitutions) are marked with vertical connecting lines. Conservative substitutions are I, V, L; Y, W, F; A, G; S, T; R, K; D, E; N, Q.

A1 (32%), A2 (36%), and A3 (37%), and with complement factors B (29%) and C2 (26%).

The vWF protein contains at least two independent collagen-binding sites, localized to the A1 and A3 domains (42, 48) and, likewise, CMP-1 and CMP-2 domains may be used to mediate interaction with collagen (1). Because VLA-2, vWF, and CMP all share related domains and can interact with collagen, that suggests the I-domain may be important for adhesion to collagen. However, the presence of an I-domain (or related sequence) does not completely correlate with a collagen adhesion function since there is no evidence that p150,95 or Mac-1 interact with collagen and, likewise, complement factors B and C2 are not known to bind collagen.

Grouping of Integrin α Subunits into Subsets

From sequence analysis, together with earlier data, it is clear that there are several structural features that distinguish integrin α subunits containing I-domains from those that do not (Table I). It has previously been noted that the mature forms of integrin α subunits α^5, α^V, and IIb each contain a cleaved peptide fragment (the COOH-terminal 15% of the protein) that is attached to the rest of the subunit by a disulfide linkage. Consistent with this, dibasic protease cleavage sites have been noted in the appropriate locations in each of those sequences (2, 35, 58). Notably (Table I), the subunits with I-domains (α^2, α^M, and p150) do not undergo protease cleavage, whereas the cleaved subunits do not have I-domains. In this regard, in the region where protease cleavage occurs for α^5, α^V, and IIb, the three I-domain subunits have gaps of 20 or more amino acids and no dibasic amino acids (see Fig. 7, α^2 position 1,018).

Figure 7. Alignment of the α-chain protein sequences of VLA-2 with other integrin α subunits. The α^2 sequence is compared to human integrin α-subunit sequences from fibronectin receptor (2, 11), gpIIb (45), vitronectin receptor (58), Mac-1 (3, 8), and p150 (7). The 191-amino acid I-domain present in α^2 (residues 159–349), as well as corresponding regions in p150 and Mac-1, have been omitted from this figure (see Fig. 2). Residues in other sequences identical to those in α^2 are boxed. In four places, short gaps appear in all six sequences. These gaps allow for maximized future alignment with VLA α^4 sequence (6la).

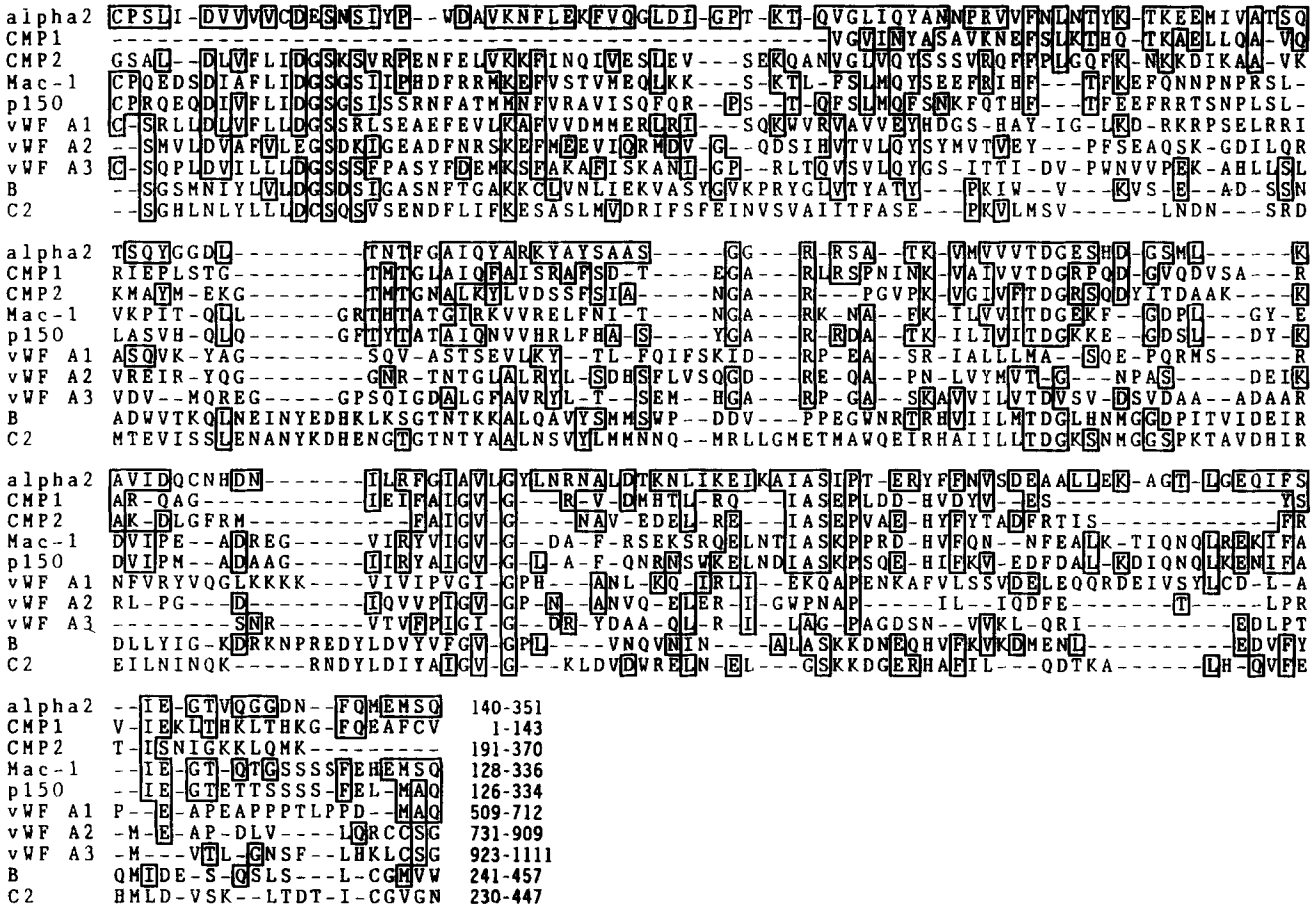

Figure 8. Comparison of the α^2 I-domain with similar domains in other proteins. CMP-1 and CMP-2 domains (1), I-domains from the α subunits of Mac-1 and p150,95 (3, 7, 8), vWF domains A1, A2, and A3 (54), and domains in complement factors B (40) and C2 (4) are compared to the I-domain from α^2 (amino acid residues 140–349). Although the actual inserted I-domain sequence in α^2 is 191 amino acids (residues 159–349), additional α^2 amino acids (residues 140–158 and 350–351) are included to accommodate similarities with other protein domains that extend beyond the 191-amino acid insert. Residues that are identical in both the α^2 sequence and other sequences are boxed.

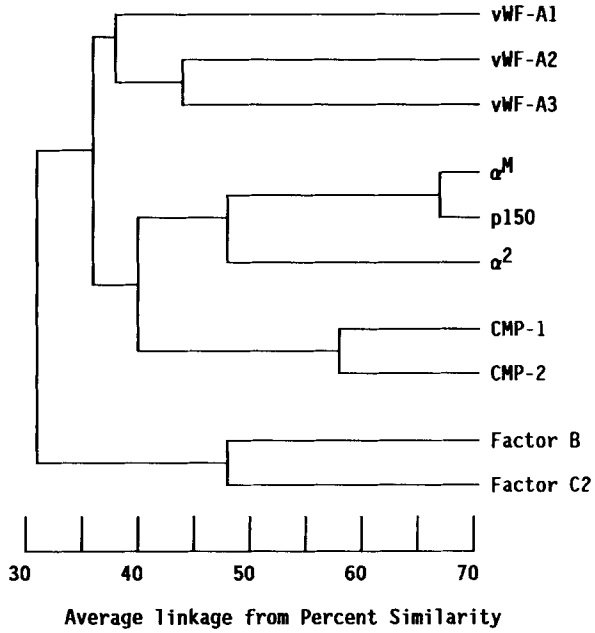

Figure 9. Linkages between α^2-subunit I-domains and similar domains in other proteins. Based on the alignment in Fig. 8, percent similarities were calculated (allowing for conserved amino acids as defined in Fig. 6), and then linkage trees were determined by standard procedures using the average linkage values (55). Similar determinations, not allowing for conserved amino acids (based on percent identical amino acids only), yielded similar results except that α^2 clustered with CMP-1 and CMP-2 instead of α^M and p150.

Another feature that differentiates between these sets of α subunits is the number of potential divalent cation sites. The cleaved subunits have four such sites (located in repeat regions IV, V, VI, and VII), whereas the I-domain subunits have only three sites (located in repeats V, VI, and VII).

Not only are there differences in the number of divalent cation sites, but evidence also suggests that the two groups of integrin α subunits may each have their own characteristic intrachain disulfide loops. Analysis of cysteines (Fig. 7) reveals that the majority (14 cysteines) are conserved throughout all six integrin α-subunit sequences. However (Table I),

Table I. Structural Features Distinguishing Integrin α Subunits with I-Domains from α Subunits That Are Cleaved

Integrin α subunit	I-Domain	Protease cleaved	Divalent cation sites	Residues at α_2 positions 110	467	350	645
α^2	Yes	No	3	Cys_{110}	Cys_{467}	Ser_{350}	$-_{645}$
α^M	Yes	No	3	Cys_{97}	Cys_{446}	Ser_{335}	Gly_{534}
p150	Yes	No	3	Cys_{97}	Cys_{444}	Ala_{333}	Val_{531}
α^5	No	Yes	4	Lys_{125}	Ala_{289}	Cys_{164}	Cys_{481}
IIb	No	Yes	4	Glu_{117}	Ala_{295}	Cys_{167}	Cys_{484}
α^V	No	Yes	4	Met_{112}	Ala_{280}	Cys_{155}	Cys_{472}
PS2α	No	Yes	4	Thr_{157}	Ala_{337}	Cys_{198}	Cys_{536}

cysteines at positions 110 and 467 in the α^2 sequence are conserved only among α^2, α^M, and p150. Conversely, at α^2 positions 350 and 645, cysteines are absent from α^2, α^M, and p150, but present in all of the cleaved subunits. Thus, it might be predicted that each group of subunits (cleaved or with I-domains) may have a characteristic intrachain disulfide loop that distinguishes one group from the other. Although the newly derived sequence for the α^L chain of LFA-1 (32) is not shown here, that sequence has an I-domain and fits the pattern shown in Table I with respect to potential divalent cation sites and conserved cysteine residues. Structural information for the *Drosophila* PS2 α subunit (6) is also included in Table I. Although the source is phylogenically far removed from humans, the properties of that α subunit are fully consistent with the properties of the other cleaved α subunits.

In addition to the "group-specific" cysteine residues mentioned in Table I, there are many other amino acids at positions throughout the aligned sequences that distinguish I-domain integrins from cleaved integrins. In fact, when the aligned sequences (Fig. 7) were searched by computer to find all positions in which exactly three sequences had identical amino acids, this was found to occur most often (at 99 positions) among the set α^5, IIb, and α^V and also occurred often (at 64 positions) among the set α^2, p150, and α^M (Table II, *part A*). Thus, the I-domain subunits could again be distinguished from the cleaved subunits, this time based on exclusively shared amino acids distributed throughout the coding region. Other sets of three subunits shared amino acids less frequently (at ≤ 24 positions) and had no obvious structural similarities. Thus the results from the data in Table II, *part A*, support the conclusions drawn from Table I.

*Table II. Comparison of Subsets of α Subunits That Show Amino Acid Identity at the Most Positions**

	Subset of α subunits	Positions with identity (No.)	Common features
Part A			
	α^5, IIb, α^V	99	Cleaved subunits
	α^2, p150, α^M	64	I-Domain
	α^5, α^2, α^V	24	–
	IIb, α^M, p150	21	–
	α^5, p150, α^M	19	–
	α^V, α^M, p150	19	–
	α^2, α^5, IIb	13	–
	α^2, IIb, α^V	13	–
Part B			
	p150, α^M	256	β_2
	α^5, α^V	109	–
	IIb, α^V	65	β_3
	α^5, IIb	62	–
	α^2, IIb	40	–
	α^2, α^V	35	–
	α^2, α^5	31	β_1

* Sets of sequences, shown in order of prevalence, were derived from computer-generated lists of positions in which only three (*part A*) or only two (*part B*) identical amino acids are shared. Sequence information from I-domains (residues 159–349 in α^2) was omitted for these determinations.

Because the α^2 and α^5 subunits both associate with the same β subunit (β_1), it might be assumed that these two sequences would show exclusive amino acid identity at several positions. However, when a computer search was performed (Table II, *part B*), positions with amino acid identity between only α^2 and α^5 were not very prevalent (seventh in the list of subunit pairs). In fact, α^2 was more likely to share residues with IIb or α^V than with α^5, and α^5 was more likely to share residues with α^V or IIb than with α^2. Thus, despite their common β-subunit association, the α^2 and α^5 subunits have relatively few amino acids common to only those sequences, making it difficult to predict potential α-subunit sites that may be specific for β_1 interaction.

Summary

Cloning and sequencing of the α^2 subunit of VLA-2 has revealed its complete primary structure, and confirmed its relatedness to other integrin α subunits. The presence of an α^2 I-domain, which possibly participates in VLA-2 adhesion to collagen has been established, and comparative studies of the α^2 sequence have revealed that subunits containing I-domains have a number of additional structural features that distinguish them from cleaved integrin α subunits. Also, it is clear that β-subunit use does not accurately predict the relative degree of similarity between integrin α subunits. For future studies, the results shown here will provide the basic information and materials needed for (*a*) additional structural comparisons with other integrins; (*b*) analysis of VLA-2 function through expression and mutagenesis; and (*c*) analysis of patient samples to measure deficient or mutated α^2.

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