Cloning of an integrin β subunit exhibiting high homology with integrin β_3 subunit

(amino acid sequence/RNA blot analysis/polymerase chain reaction/vitronectin receptor)

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ABSTRACT cDNAs for another β subunit of the integrin family were isolated with the aid of polymerase chain reaction and sequenced. The combined cDNA sequence is 3110 base pairs (bp) in size and has one long open reading frame of 2388 bp. The deduced amino acid sequence is similar to those of other integrin β subunits but does not correspond to β_1 , β_2 , β_3 , or β_4 subunits. This β subunit is divided by a membrane-spanning domain into a large extracellular domain at the N-terminal side and a small intracellular domain at the C-terminal side. The extracellular domain has a cysteine-rich region that contains four repeats of 8-cysteine motifs. All 56 cysteine residues found in the extracellular domains of other mature β subunits are present in this β subunit. The β subunit reported here has particularly high homology with the β_3 subunit. The mRNA for the molecule is ≈ 3.5 kbp in size and is expressed in various cell types. Other researchers have recently reported additional β subunits that associate with the vitronectin receptor α subunit. The deduced amino acid sequence of this molecule contains the N-terminal partial amino acid sequence of one of these β subunits, β_x . The β subunit described herein seems to be identical to the β_x subunit and to function as the β subunit of a vitronectin receptor.

Integrins are cell-surface receptors for extracellular matrix molecules and are composed of two distinct noncovalently associated subunits, the α and β subunits. More than 10 different α subunits have been described, but, until recently, only 3 different β subunits were known to exist (1–3). However, recent studies have suggested that three, and possibly four, additional β subunits are expressed in various cells (4–9). One of these β subunits, called β_4 , has a larger molecular size (≈ 190 kDa) than do other β subunits (≈ 90 kDa) and associates with the α_5 subunit (4, 8). β_4 is expressed primarily in epithelial cells. The other integrin β subunits recently reported form complexes with vitronectin receptor α subunit, α_v (5, 6, 9). Three separate groups reported such integrin subunits, but whether those molecules are identical or are distinct from one another is unknown. The third possibly different β subunit forms the mouse Peyer's patch-specific lymphocyte-homing receptor (7). This subunit, which appears different from the β_1 subunit, forms a complex with α_4 subunit, but further details have not been reported.

To characterize some of the recently identified integrin β subunits, we have cloned additional β -subunit cDNAs by using polymerase chain reaction (PCR). We have recently reported the sequence of the β_4 -subunit cDNA obtained in this research (10). Here, we describe the cloning of a discrete integrin β subunit that has high homology with the β_3 subunit.[§]

MATERIALS AND METHODS

Cloning of Partial cDNA for Additional Integrin β Subunit by PCR. Total RNA preparations were made by a guanidium isothiocyanate method (11) from human DLD-1 adenocarcinoma cells, COLO 205 adenocarcinoma cells, A 431 epidermoid carcinoma cells, K-562 myelogenous leukemia cells, U-937 histiocytic lymphoma cells, SK-N-SH neuroblastoma cells, U-251 glioma cells, and retinal pigment epithelium cells. Poly(A)⁺ RNAs were isolated with oligo(dT)-cellulose spin columns obtained from Pharmacia-LKB. The template cDNAs were synthesized from the poly(A)⁺ RNA preparations by using a cDNA synthesis kit obtained from Boehringer Mannheim.

PCR was done as described by Gould *et al.* (12), with some modifications. Two highly conserved amino acid sequences were chosen by comparing the sequences of various integrin β subunits (13–19), and two mixed oligonucleotide primers were made that contained all possible nucleotide combinations corresponding to the sequences, except for leucine 145 (Fig. 1). *EcoRI* linkers were added at the ends to facilitate the subsequent procedures. One hundred nanograms of cDNA was dissolved in 50 μ l of *Thermus aquaticus* (*Taq*) DNA polymerase buffer. After adding 100 μ l of mineral oil, 2 units of *Taq* DNA polymerase were added, and cycle reaction was initiated. The equipment used was a Perkin-Elmer/Cetus DNA thermal cycler. Denaturation occurred at 94°C for 1.5 min, hybridizing was at 55°C for 2 min, and polymerization was at 72°C for 3 min. The reaction cycle was repeated 60 times, with addition of 2 units of *Taq* DNA polymerase after 30 cycles. After completion of the PCR, the products with an expected size of ≈ 300 –350 base pairs (bp) were separated by agarose gel electrophoresis, extracted, and digested with *EcoRI*. The resultant DNAs were subcloned into M13 vector, and white clones containing inserts were obtained after transformation in the presence of isopropyl β -D-thiogalactoside and 5-bromo-4-chloro-3-indolyl β -D-galactoside.

Screening of Placental cDNA Library and DNA Sequencing. About 7.5×10^5 plaques of a placental cDNA library obtained from J. L. Millán (20) were screened by the plaque-hybridization method by using as probe the partial cDNA obtained from PCR experiments. The positive plaques were plaque-purified. The inserts were then cut out with *EcoRI* and subcloned into M13 and pGEM1 vectors for further analysis.

DNAs subcloned into M13 vector were sequenced by using the dideoxynucleotide chain-termination method of Sanger *et al.* (21). The Sequenase DNA sequencing kit (United States Biochemical) and adenosine [³⁵S]thiotriphosphate (New En-

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Abbreviation: PCR, polymerase chain reaction.

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[§]The sequence reported in this paper has been deposited in the GenBank data base (accession no. M35011).

FIG. 1. Amino acid sequences and corresponding oligonucleotide sequences used for PCR primers. Amino acid sequences 1 and 2 were chosen by comparing various integrin β subunits (13–19). Numbering of sequences 1 and 2 was taken from that of corresponding amino acid sequences in the human integrin β_1 sequence (14). The nucleotide sequence is as follows: A, deoxyadenosine; C, deoxycytosine; G, deoxyguanosine; T, deoxythymidine; R, either A or G; Y, either C or T; N, either A, C, G or T. +, The sequences of CTY were included because the amino acid residue at this position of β_2 subunit is leucine. *, The sequences TTR were not included because the known integrin β subunits use only sequences CTN for the leucine residue.

gland Nuclear) were used. The DNA sequences described herein were from the sequencing of both strands.

Other Procedures. Cells were cultured in Dulbecco's modified Eagle's medium or RPMI 1640 medium supplemented with 10% fetal calf serum.

DNA was radiolabeled with [32 P]dCTP by using a random-primed DNA labeling kit obtained from Boehringer Mannheim. mRNA preparations were run on 0.8% agarose containing 2.2 M formaldehyde and 0.02 M sodium acetate (22), and RNA blotting analyses were done according to the method of Thomas (23). DNA and amino acid sequences were analyzed by using the MicroGenie program obtained from Beckman.

RESULTS

Cloning of Another β Subunit. We used a PCR approach (12) to clone cDNAs for integrin β subunits. Eight different template cDNAs were used for PCR, and six to eight white clones from each template cDNA were obtained and sequenced. Approximately two-thirds of the white clones had inserts of which deduced amino acid sequences were not homologous with those of integrin β subunits. Among the remaining 18 clones, 14 clones from various template cDNAs contained β_1 subunit sequences; one clone from U-937 template cDNA had β_2 sequence and one clone from the COLO 205 template cDNA corresponded to the possible β_4 subunit (10). The remaining three clones, one each from DLD-1, K-562, and SK-N-SH template cDNAs, contained an insert sequence significantly homologous with, but not identical to, those of known integrin β subunits. One of these inserts (m β 5-D2) was used to screen a placental cDNA library as probe to obtain the entire coding sequence of the molecule. Nine positive clones were isolated from $\approx 7 \times 10^5$ plaques screened and sequenced.

Amino Acid Sequence of the Additional β Subunit. The combined cDNA sequence is 3110 bp long and contains one long open reading frame of 2388 bp following the first AUG codon (Fig. 2). The AUG codon is within the sequence that matches the consensus sequence of the translation initiation site described by Kozak (24). The deduced amino acid sequence (796 amino acids) clearly shows that the molecule defined by the cDNA is an additional integrin β subunit. We have tentatively named this molecule the β_5 subunit, because this subunit is not the β_1 , β_2 , β_3 , or β_4 subunit (see *Discussion*). However, its gross structure is very similar to other integrin β subunits. One putative membrane-spanning domain located near the C terminal divides the molecule into a large extracellular domain at the N-terminal side and a small cytoplasmic domain at the C-terminal side. The extracellular domain has a cysteine-rich region that contains four repeats of an 8-cysteine motif (13). The β_5 subunit contains all 56 cysteine residues found in the extracellular domains of var-

ious other mature β subunits (13–19). The cytoplasmic domain contains a possible tyrosine phosphorylation site, as has been described in β_1 and β_3 subunits (13, 15). The β_5 subunit has eight possible N-linked glycosylation sites in the extracellular domain and two sites in the cytoplasmic domain.

Comparison of the β_5 subunit with other integrin β subunits shows that β_5 has high homology with β_3 subunit; $\approx 55\%$ of the amino acids are identical when these two sequences are aligned (Fig. 3), which is higher than comparisons with the various other human β subunits.

RNA Blot Analysis. Northern (RNA) blot analysis with a β_5 cDNA probe showed a 3.5-kbp band in almost all human cells examined at various levels (Fig. 4A). Some bands were very faint (Fig. 4A, lanes 3–5 and 7), but longer exposure clearly revealed their presence. A similar band was detected also in the mRNA of rat and mouse cells. On the other hand, the mRNA for the highly homologous β_3 subunit was detected in the mRNA preparations from some, but not all, human lines (Fig. 4B). Moreover, it was not detected in preparations made from various rat or mouse cells and tissues we examined, even under low-stringency washing conditions.

DISCUSSION

The molecule defined by the present cDNAs is another β subunit of the integrin family. The deduced amino acid sequence clearly shows that this subunit (tentatively named β_5 subunit) is not identical to the β_1 , β_2 , or β_3 subunit. Recently, Sonnenberg *et al.* (4) and Kajiji *et al.* (8) reported an additional β subunit, called β_4 subunit, which is also dissimilar, because the β_5 sequence does not contain the N-terminal amino acid sequence reported for β_4 subunit (8, 25). The β_5 sequence differs from the sequence deduced recently from the β_4 subunit cDNA clones (10). Holzmann *et al.* (7) reported possibly another β subunit in murine lymphoma cells that binds specifically to mouse Peyer's patches. This molecule has not been characterized well, but expression may be limited to lymphocytic cells. Therefore, the β_5 subunit is probably not the human homologue of that molecule.

Cheresh *et al.* (5), Freed *et al.* (6), and Krissansen *et al.* (9) have reported other integrin β subunits, called β_x , β_s , and β_{3b} , respectively, which make complexes with the vitronectin receptor α subunit, α_v . Details of these molecules have not yet been reported. However, Smith *et al.* recently obtained the N-terminal 10-amino acid sequence of β_x subunit (D. A. Cheresh, personal communication) (32). The deduced amino acid sequence of β_5 subunit contains that sequence (amino acids 25–34) following the putative signal sequence, which strongly suggests that the β_5 and β_x subunits are identical. It is unclear, however, whether β_s and β_{3b} subunits are the same as or distinct from the β_5 subunit. The β_3 subunit seems to be expressed in many different cell types (6), as is the case for the β_5 (Fig. 4A) and β_x subunits (5). This raises the possibility

Freed *et al.* (6) reported that a serine residue in the cytoplasmic domain of the β_5 subunit, unlike the β_3 subunit, was phosphorylated by protein kinase C when MG-63 cells were treated with the phorbol-ester tumor promoter phorbol 12-myristate 13-acetate, and they suggested that the phosphorylation might be involved in regulation of integrin function. The β_5 subunit that seems to be very similar or identical to the β_s subunit contains five serine residues in the cytoplasmic domain, which suggests the possibility that the β_5 subunit is a substrate for the protein kinase C. To clarify this, it will be important to examine whether protein kinase C can phosphorylate the oligopeptides corresponding to the sequences containing these serine residues *in vitro*.

FIG. 3. Comparison of the deduced amino acid sequence of human β_5 subunit with that of β_3 subunit. Identical residues are boxed. Dashes denoting gaps are introduced to maximize the homology. The putative membrane-spanning domain is underlined.

Although most cultured cells adhere to vitronectin substratum, the expression of β_3 subunit is not so evident as that of α_v subunit in many cells (Fig. 4B). Specifically, the mRNAs for mouse or rat β_3 subunit were not detected in our studies, even under conditions of low stringency. If β_5 and β_x subunits are the same molecule, then the β_5 subunit would form a complex with α_v subunit, and the resultant receptor would be expected to have vitronectin-binding activity. If this is the case, adhesion to vitronectin may be attributable, at least in part, to the $\alpha_v\beta_5$ complex in many cells. Moreover, $\alpha_v\beta_x$ and possible $\alpha_v\beta_5$ complex seems to have a ligand specificity different from that of the $\alpha_v\beta_3$ complex (5, 6, 28). The fact that some cells have both types of receptors and that both receptors can be purified by the same purification protocol (5, 6, 28) may explain, in part, the difference in ligand specificity of the vitronectin receptor from various sources (5, 29–31). The availability of a full-length cDNA for β_5 subunit will allow examination of these questions by gene transfer.

It would be interesting to know how many β subunits exist in the integrin family. In the present study, we examined ≈ 70 clones derived from various cDNA preparations but could isolate clones for only four different β subunits among five or possibly six β subunits. This indicates that the isolation of clones corresponding to the β subunits that are expressed at relatively low level, and/or that are expressed in only certain cell types, is difficult by the simple procedure we used. This result suggests that no other β subunit is expressed at high levels in the cell types used. This finding does not necessarily rule out the possibility that other, as yet unknown, β subunits are expressed in a limited number of cell types and/or at specific developmental stages, nor does it rule out the possibility that there exists even another type of β subunit that lacks the sequences corresponding to primers 1 and 2.

The present study identified an additional integrin β subunit that may form a specific vitronectin receptor. This molecule may prove useful for studying the biological functions of the complex integrin family.

FIG. 4. RNA hybridization analysis of integrin β_5 subunit. Total RNA preparations were separated electrophoretically on agarose gel containing formaldehyde, transferred to nitrocellulose filters, and probed with a β_5 subunit cDNA (m β_5 -D2) (A) and β_3 subunit cDNA (2.2-kbp *Eco*RI fragment) (B). Calf liver 28S (4.7 kb) and 18S (1.9 kb) ribosomal RNAs were used as size markers. Lanes: 1, IMR-90 diploid fibroblasts; 2, MG-63 osteosarcoma cells; 3, K-562 myelogenous leukemia cells; 4, U-937 histiocytic lymphoma; 5, COLO 205 colon adenocarcinoma cells; 6, DLD-1 colon adenocarcinoma cells; 7, A 431 epidermoid carcinoma cells; 8, retinal pigment epithelium cells; 9, SK-N-SH neuroblastoma cells; 10, U-251 glioma cells.

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