cDNA Cloning and Chromosomal Localization of Human α₁₁ Integrin

A COLLAGEN-BINDING, I DOMAIN-CONTAINING, β₁-ASSOCIATED INTEGRIN α-CHAIN PRESENT IN MUSCLE TISSUES*

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We previously identified a novel integrin α-chain in human fetal muscle cells (Gullberg, D., Velling, T., Sjöberg, G., and Sejersten, T. (1995) Dev. Dyn. 204, 57–65). We have now isolated the full-length cDNA for this integrin subunit, α₁₁. The open reading frame of the cDNA encodes a precursor of 1188 amino acids. The predicted mature protein of 1166 amino acids contains seven conserved FG-GAP repeats, an I domain with a metal ion-dependent adhesion site motif, a short transmembrane region, and a unique cytoplasmic domain of 24 amino acids containing the sequence GFRRS. α₁₁, like other I domain integrins, lacks a dibasic cleavage site for generation of a heavy chain and a light chain, and it contains three potential divergent cation binding sites in repeats 5–7. The presence of 22 inserted amino acids in the extracellular stalk portion (amino acids 804–826) distinguishes the α₁₁ integrin sequence from other integrin α-chains. Amino acid sequence comparisons reveal the highest identity of 42% with the α₁₀ integrin chain. Immunoprecipitation with antibodies to α₁₁ integrin captures a 145-kDa protein distinctly larger than the 140-kDa α₂ integrin chain when analyzed by SDS-polyacrylamide gel electrophoresis under nonreducing conditions. Fluorescence in situ hybridization maps the integrin α₁₁ gene to chromosome 15q23, in the vicinity of an identified locus for Bardet-Biedl syndrome. Based on Northern blotting, integrin α₁₁ mRNA levels are high in the adult human uterus and in the heart and intermediate in skeletal muscle and some other tissues tested. During in vitro myogenic differentiation, α₁₁ mRNA and protein are up-regulated. Studies of ligand binding properties show that α₁₁β₁ binds collagen type I-Sephrose, and cultured muscle cells localize α₁₁β₁ into focal contacts on collagen type I. Future studies will reveal the importance of α₁₁β₁ for muscle development and integrity in adult muscle and other tissues.

Integrins are heterodimers composed of noncovalently associated α- and β-chains that connect cells to the extracellular matrix or to other cells (1). In addition to acting as mechanical links between the cytoskeleton and extracellular ligands, integrins are signal-transducing receptors that influence processes such as cell proliferation, cell migration, and cell differentiation (2–4). Integrins can be grouped into subfamilies based on shared β-chains, shared ligand binding properties, or shared structural features of the α-chains. Currently, 17 α-chains and 8 β-chains have been identified (5). Of the subfamilies with shared β-chains, the β₁ subfamily has the most members. To date, 11 integrin α-chains associated with the β₁ chain have been identified and characterized, α₁–α₁₀ and α₁₁ (5).

Several integrins bind the sequence RGD in their respective ligands (1). Of those integrins identified thus far, α₅, α₆, α₇, α₉, α₁₀, α₁₁, α₁₂, and α₁₃-chains form heterodimers that mediate RGD-dependent interactions. The ligands containing RGD are generally found in the interstitial type of extracellular matrix. Major non-RGD-dependent ligands include various collagens and laminin isoforms. Although both collagens and laminins contain the RGD sequence in their primary sequences, these RGD sequences are cryptic (6–9) and are not normally accessible to cells in the native proteins, but they may be exposed during growth and reorganization events of the extracellular matrix.

Another subdivision of integrins can be made based on structural similarities of the α-chains. A number of integrins contain an extracellular (10, 11) that is homologous to collagen binding present in von Willebrand factor (12). The I domain constitutes an inserted domain of approximately 200 amino acids that is present in eight known integrins (α₁, α₂, α₅, α₆, α₇, α₉, α₁₀, and α₁₁) (5, 10). Structural analysis of integrin I domains crystallized in the presence of Mg²⁺ have revealed the presence of a characteristic metal ion-dependent adhesion site motif shown to be critical for ligand binding (13). Integrin α-chains containing the I domain are not cleaved into heavy and light chains, although the α₅ chain possesses a proteolytic cleavage site near the membrane-spanning region (14, 15). For I domain integrins, the principal ligand binding sites are found within the I domain (10). Known ligands for I domains found within the β₁ integrin subfamily include laminins and collagens (α₁β₁ and α₂β₁ integrins) (16–19), and Echovirus (α₂β₁ integrin) (20).

Structure comparisons have suggested that integrins fold into a so-called seven-bladed β-propeller structure that forms one globular domain with the ligand binding region on the upper surface (21). The I domain is inserted between blades 2 and 3 in this propeller, and divergent cation binding sites are located on the lower surface in blades 4–7 (22, 23). Studies of β₂ integrins have revealed that proper folding of the β₂-chain is dependent on the presence of the α₂-chain, but that the I domain folds independently of other structural elements in the
α- and β-chain (24). In integrin α-chains, a less conserved stalk region separates the predicted β-propeller from the short transmembrane region. This stalk region may possibly be involved in transducing conformational changes between the extracellular and intracellular regions, as well as mediating protein-protein interactions. Although integrins take part in cell signaling events, the cytoplasmic tail is short and lacks enzymatic activity. The sequence GFFKR is conserved in a majority of integrin α-subunit cytoplasmic tails and has been shown to be important for calreticulin binding (25).

Cellular interactions with the extracellular matrix during muscle formation and in muscular dystrophy have received increased interest during the past years. In the early 1960s, a mutant was described in Drosophila that was characterized by the detachment of muscles from their attachment points at the time of the first embryonic muscle contraction, causing the embryos to assume a spheroid shape (26). The mapping of the molecular defect in the lethal myospheroid mutant in 1988 to an integrin β-chain (27) was the first evidence for a role of integrins in muscle integrity. More recently, refined analysis of Drosophila mutants has indicated distinct roles for integrins in muscle end point attachments and sarcomere structure (28). The Drosophila integrins are all cleaved α-chains and share many features with vertebrate integrins such as the ability to cluster into focal contacts (29).

The finding that inactivation of the α2 integrin gene in the mouse (30) and mutations in the human ITGA7 gene (31) both cause muscular dystrophy, affecting mainly muscle attachment points, indicates a striking conservation of integrin function during evolution. Of the 11 members of the β subfamily, α7 exists as a major integrin α-chain (32, 33) associated with the β1D integrin chain in the adult skeletal muscle sarcolemma (34). Intriguingly, mutations in the basement membrane protein laminin α2-chain (35–37) cause a more severe disease than that observed for the laminin receptor integrin α2-β1 (30). This indicates that other receptors for laminin exist in muscle.

We recently identified a novel integrin on cultured human fetal muscle cells (38). In the current study, we describe the cloning and characterization of this novel I-domain-containing, β1-associated integrin chain, which is expressed in muscle tissues.

**EXPERIMENTAL PROCEDURES**

**Cell Cultures**—The human fetal myoblast/myocyte cultures were derived from clone G6 originating from a thigh muscle of a 73-day-old aborted fetus (Ref. 39; referred to as G6 hereafter). Cultures of G6 and 2.5-year postnatal human satellite XXVI cells, a gift from Dr. Helen Blau (Stanford University, Stanford, CA), were grown as reported previously (39). Total RNA from G6 and XXVI cells and 10 μg of total RNA from RD and A204 cells and a Human Multiple Tissue Northern blot containing poly(A) RNA from adult human tissues (CLONTECH) were hybridized at 68 °C in Human Multiple Tissue Northern solution (CLONTECH) with probes labeled as described above. The probes were labeled with [α-32P]dCTP using Ready-To-Go DNA labeling beads (Amersham Pharmacia Biotech). Three clones (Fig. 1, 1.1.1–1.3) representing parts of α2 mRNA were obtained. Rescreening of the human uterus 5′-stretch agt11 cDNA library with probe J290 (corresponding to nucleotides 2183–2473 in Fig. 1) yielded three more clones (Fig. 1, 2.1–2.3) covering the rest of α2 mRNA. Positive clones were plaque-purified, and the phage DNA was isolated using the Lambda Midi kit (Qiagen) and then subcloned into the Bluescript SK or pUC19 plasmid vectors before sequencing.

**Northern Hybridization**—A filter containing 6 μg of the poly(A) RNA from G6 and XXVI cells and 10 μg of the total RNA from RD and A204 cells and a Human Multiple Tissue Northern blot containing poly(A) RNA from adult human tissues (CLONTECH) were hybridized at 68 °C in Human Multiple Tissue Northern solution (CLONTECH) with probes labeled as described above. The probes were labeled with [α-32P]dCTP using Ready-To-Go DNA labeling beads (Amersham Pharmacia Biotech).

**cDNA Sequencing and Sequence Analysis**—All PCR fragments and cDNA clones were sequenced on both strands either manually (29) or using an ABI 310 Genetic Analyzer automatic sequencer. Sequences were analyzed with the aid of the MacVector™ and DNA Star, Autoseq™ software packages. Distance tree of all I domain-containing α1 integrins was produced against the peptide CRRPGLDPTPKVLE from the integrin α1 cytoplasmic domain. Peptide synthesis and conjugation to keyhole limpet hemocyanin, immunization of rabbits, and affinity purification were performed at Innovagen AB (Lund, Sweden). The monoclonal antibody MAB 13 (clone FB12, sold as MAB 1973) was produced against the peptide CY3™-coupled goat anti-rabbit IgG and FITC-coupled goat anti-mouse IgG of multiple labeling grade were from Jackson ImmunoResearch Laboratories, Inc. (West Grove, PA).

**Immunoprecipitation and SDS-PAGE**—G6 and XXVI cells were labeled with [35S]cysteine/methionine and subjected to immunoprecipitation and SDS-PAGE as reported previously (38). The two-step procedure was performed according to the manufacturer’s instructions (Marathon cDNA Amplification kit; CLONTECH) using cDNA prepared from G6 mRNA and the gene-specific antisense primer 5′-CTTGGGACACCTG-AAATTTGAGTTGACG-3′. Amplification was carried out applying the touch-down program (see above). To identify relevant products, 10 μl of each RACE product were resolved on a 1% agarose gel and subjected to Southern blot analysis as described previously (40). PCR2 (see above) was labeled with [α-32P]dCTP (Amersham Pharmacia Biotech) using the RedyprimeII DNA labeling system (Amersham Pharmacia Biotech) and used as a hybridization probe. One specific signal was detected.

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equilibrated in solubilization buffer. After a 1-h incubation, the column activated Sepharose CL-4B at 3 mg/ml gel as described previously (14), type I from Vitrogen (Collagen Corp., Palo Alto, CA) coupled to CNBr-proteins were applied to a collagen type I-Sepharose (bovine collagen were solubilized in 1 ml of solubilization buffer (10 mM Tris-HCl, pH 7.4, 0.15 M NaCl, and 1% Triton X-100 were added to a final volume of 1 ml, and the lysate was incubated with GammaBind G-Sepharose for 1 h. The incubation with GammaBind G was performed to ensure that no reactive β3 antibodies remained. After the removal of GammaBind G-Sepharose, α1, integrin antibody was added for an additional 2 h, followed by capture with protein A-Sepharose (Amersham Pharmacia Biotech) and boiling in SDS-PAGE sample buffer.

Chromosomal Localization—Chromosomal localization of the human integrin α11 was performed by using a combination of the fluorescence in situ hybridization (FISH) technique and 4'-diaminodino-2-phenylindole banding essentially as described previously (42). The 1.4-kb reverse transcription-PCR product PCR3 was used as a hybridization probe. Surface Iodination and Affinity Chromatography—Cultured XXVI cells were surface-iodinated as described previously (38). Labeled cells were solubilized in 1 ml of solubilization buffer (10 mM Tris-HCl, pH 7.4, 15 mM NaCl, 1% Triton X-100, 1 mM MgCl2, 1 mM CaCl2, and 1 mM MnCl2) and centrifuged at 14,000 × g for 20 min, and soluble membrane proteins were applied to a collagen type I-Sepharose (bovine collagen type I from Vitrogen (Collagen Corp., Palo Alto, CA) coupled to CNBr-activated Sepharose CL-4B at 3 mg/ml gel as described previously (14)), equilibrated in solubilization buffer. After a 1-h incubation, the column was washed extensively with buffer A (10 mM Tris-HCl, pH 7.4, 50 mM NaCl, 1 mM MgCl2, 1 mM CaCl2, and 1 mM MnCl2) and centrifuged at 14,000 × g for 20 min, and soluble membrane proteins were applied to a collagen type I-Sepharose (bovine collagen type I from Vitrogen (Collagen Corp., Palo Alto, CA) coupled to CNBr-activated Sepharose CL-4B at 3 mg/ml gel as described previously (14)), equilibrated in solubilization buffer. After a 1-h incubation, the column was washed extensively with buffer A without NaCl. Bound proteins were eluted with 20 mM EDTA, 10 mM Tris-HCl, pH 7.4, and 0.1% Triton X-100. Peak fractions were pooled and concentrated by immunoprecipitation with β1 integrin and α11 integrin antibodies as described under “Immunoprecipitation and SDS-PAGE.” Eluted fractions and captured proteins were analyzed on 7.5% SDS-PAGE gels, followed by autoradiography. Indirect Immunofluorescence—Cells cultured on coverslips were washed in serum-free medium and fixed for 8 min in acetone at −20 °C. Nonspecific binding sites were blocked by incubating with 10% goat serum diluted in phosphate-buffered saline. In the double immunofluorescence staining protocol, primary antibodies (anti-α11, cyt (rabbit antibody) and anti-vinculin (mouse antibody)) were simultaneously incubated with fixed cells for 1.5 h at +37 °C. Specifically bound antibodies were detected using anti-rabbit Cy3 IgG and anti-mouse FITC IgG. Stained cells were mounted in Vectashield™ mounting medium (Vector Laboratories Inc., Burlingame, CA) and visualized and photographed under a Zeiss light microscope equipped with optics for observing fluorescence.
It will be interesting to determine the importance of this sequence in defining the cytoplasmic domain as well as its possible ability to bind calreticulin and other intracellular components.

Comparison of Integrin \( \alpha_{11} \)-Chain with Other Integrin \( \alpha \)-Chains—Alignment of the predicted \( \alpha_{11} \) integrin amino acid sequence with other integrin sequences shows the highest overall identity with \( \alpha_{10} \) (42% identity), \( \alpha_1 \) (37% identity), and \( \alpha_2 \) (35% identity), followed by the remaining I domain-containing integrin subunits. Of the non-I domain-containing integrins, \( \alpha_4 \) and \( \alpha_9 \) are the most similar to \( \alpha_{11} \). A distance tree shows that \( \alpha_{10} \) and \( \alpha_{11} \) form a separate branch from the most closely related \( \alpha_1 \) and \( \alpha_2 \) integrin chains (Fig. 3). The similarity with other integrins is particularly high in the amino-terminal \( \beta \)-propeller part but is lower in the stalk region. Comparison of \( \alpha_1 \) integrin with \( \alpha_2 \) integrin has pointed to the presence of a 38-residue insert in the \( \beta \)-propeller region of the \( \alpha_1 \) integrin chain (15). Like the \( \alpha_1 \) chain, \( \alpha_{11} \) also contains inserted amino acids not present in the other I domain-containing integrin chains. However, in the \( \alpha_{11} \) chain, these are found within the stalk region at amino acids 804–826. The exact border of the predicted insertion varies depending on the alignment method and the parameters chosen, but it is predicted to span at least 22 amino acids. The insert contains two cysteines and shows no significant similarity to other integrin sequences (see Fig. 2).

We do not believe that the predicted inserted sequence represents a cloning artifact because it is present in three independently analyzed clones. Other examples of non-I domain inserted sequences are found in the \( \alpha_7 \) integrin chain, in which developmentally regulated splicing in the ligand binding region modulates ligand affinity (46). In the \( \alpha_7 \) integrin chain, splicing...
in the extracellular domain between predicted blades 2 and 3 in the β-propeller generates the X1 and X2 variants, affecting the binding to laminin-1 in a cell-specific manner (47). In the more closely related α₃ integrin chain, the 38 extra amino acids are present in a position that is predicted to be in the beginning of the sixth blade of the seven-bladed propeller. Thus far, there is no evidence that the extra amino acids in either α₁ or α₁₁ arise by alternative splicing. In α₁₁, the predicted inserted region is outside the β-propeller and most likely does not directly affect ligand binding. It is nevertheless interesting to note that by binding to the stalk region of certain integrin α-chains, tetraspan proteins can recruit phosphatidylinositol 4-kinase and protein kinase C to integrin complexes (48). Likewise the extracellular membrane-proximal parts of certain integrin α-chains have been shown to be involved in Shc-mediated integrin signaling (49).

Analysis of the sequences identified during screening for genes up-regulated during tadpole regression revealed a partial sequence, which at the time was reported to show the highest similarity to integrin α₁ (41% identity) (50). This sequence, when translated (amino acids 1–116), shows 71% identity to human α₁₁ and thus most likely represents the Xenopus orthologue of α₁₁ rather than that of the α₁. These data suggest that α₁₁ is well conserved during evolution.

Chromosomal Localization of the Integrin α₁₁ Gene—A fluorescent cDNA probe was used for in situ hybridization on metaphase chromosome spreads. The analysis shows that the integrin α₁₁ gene (ITGA11) is located on chromosome 15q23 (Fig. 4). The genes for I domain-containing integrins α₁ and α₂ are both present on chromosome 5 (51, 52), just as the genes for the closely related β₂ integrin-associated α-chains all map to chromosome 16 (53). Interestingly, the α₁₁ gene and the closely related α₁ and α₂ genes map to different chromosomes. It will be of evolutionary interest to determine the chromosomal localization of the integrin α₁₀ gene. Curiously, a form of Bardet-Biedl syndrome characterized by retinitis pigmentosa, polydactyly, obesity, hypogonitalism, mental retardation, and renal anomalies maps to 15q22–23 (54).

Expression Pattern of α₁₁ mRNA in Adult Tissues—Northern blot analysis of mRNA from various adult human tissues shows the highest level of expression of α₁₁ in the adult human uterus. A strong signal is also noted in the heart, whereas intermediate levels of α₁₁ mRNA are present in skeletal muscle, and intermediate to low levels are present in other adult nonmuscle tissues such as the pancreas, kidney, and placenta (Fig. 5; data not shown). For a comparison, the same blot was probed for the closely related α₁ integrin mRNA (Fig. 5). A striking difference in the expression levels of α₁ and α₁₁ was observed in the smooth muscle-rich uterus, which appears to lack α₁. Immunohistochemical analysis and in situ hybridizations will elucidate the detailed distribution of α₁₁ protein and mRNA in muscle and other tissues. Neither α₁ (33) nor α₂ (55) is present in muscle fibers, and the distribution of α₁₀ in skeletal muscle tissues is not known (5). Hence, no I domain-containing integrin has yet been reported to be expressed in the skeletal muscle sarcolemma. Recently, the gene for α₁ integrin was inactive in mice, resulting in mice with an apparently normal phenotype (56). More careful analysis revealed a phenotype characterized by a hypocellular skin (57) and aberrant regulation of collagen synthesis (58). It will be interesting to compare sites of overlapping expression among α₁, α₂, and α₁₁ integrins and to use reagents to α₁₀ and α₁₁ to examine possible functional compensatory mechanisms in α₁ integrin-deficient mice.

Biochemical Characterization of α₁₁ Protein—After the cloning of the full-length α₁₁ integrin cDNA, it was essential to determine whether the predicted amino acid sequence was identical to the novel uncleaved β₁ integrin-associated α-chain that we had previously noted to be up-regulated during in vitro differentiation of human myoblasts (38). To answer this question, we raised antibodies to the cytoplasmic tail of the integrin

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**Fig. 4.** Chromosome mapping of the ITGA11 gene by FISH. A, the left panel shows the FISH signals on human chromosome 15; the right panel shows the same mitotic figure stained with 4',6-diamino-2-phenylindole to identify human chromosome 15. B, diagram of the FISH mapping result for probe PCR3 based on a detailed analysis of 10 different images. Each dot represents the double FISH signals detected on human chromosome 15.

**Fig. 5.** Expression of integrin α₁₁ and α₁ subunit mRNAs in adult human tissues. Integrin α₁₁ mRNA and integrin α₁ mRNA were analyzed on a membrane with RNA from various adult human tissues where mRNA loading was normalized with respect to β-actin. Probes used for hybridizations are marked on the left. The size standard is marked to the right. Note that the β-actin probe reacts with 2 kb of β/γ-actin transcripts and the muscle-specific 1.8-kb α-actin message.
Cloning of $\alpha_{11}$ Integrin Chain

$\alpha_{11}$ chain. Immunoprecipitation from the human satellite cells showed that the antibodies precipitated a 145-kDa $\alpha_{11}$ band associated with a 115-kDa $\beta_1$ band (Fig. 6A) in SDS-PAGE under nonreducing conditions. Under reducing conditions, the $\alpha_{11}$ band migrated as 155 kDa (see Fig. 6B). From the translated amino acid sequence, a $M_t$ of 133,400 is predicted for the $\alpha_{11}$ chain. Taking the 16 potential glycosylation sites into account, this fits well with the observed 155-kDa band in SDS-PAGE. Under nonreducing conditions, the 145-kDa band is distinctly larger than $\alpha_2$ (Fig. 6A) and $\alpha_{10}$ integrin chains that co-migrate as 140-kDa bands, and $\alpha_{11}$ migrates well below the 180-kDa integrin $\alpha_1$ band. The $\alpha_2$ (59) and $\alpha_{10}$ (5) integrin chains both contain 10 potential glycosylation sites, whereas $\alpha_1$ contains 26 glycosylation sites (60). The intermediate size of $\alpha_{11}$ in SDS-PAGE compared with $\alpha_1$ and $\alpha_2/\alpha_{10}$ is thus most likely a result of differential glycosylation.

To show that $\alpha_{11}$ is associated with the $\beta_1$-subunit, we performed a two-step immunoprecipitation procedure. Integrins were first precipitated with a monoclonal anti-$\beta_1$ integrin antibody, and GammaBind G-captured integrins were then dissociated by boiling in 1% SDS. In the second step, SDS was diluted tenfold, and antibodies to $\alpha_{11}$ were added. As shown in Fig. 6A, antibodies to $\alpha_{11}$ immunoprecipitate only the 145-kDa band from the dissociated precipitate initially captured with $\beta_1$ antibodies.

Induction of $\alpha_{11}$ mRNA and Protein during Myogenic Differentiation in Vitro—We have previously determined that $\alpha_{2\text{mt}}$ is the major integrin $\alpha$-chain that is up-regulated during myogenic differentiation on human fetal myoblasts in vitro (38). To compare $\alpha_1$ levels in myoblasts and myotubes, immunoprecipitates were analyzed from myoblast cultures in proliferation medium and from parallel cultures allowed to differentiate for 7 days. Immunoprecipitation with both $\beta_1$ and $\alpha_{11}$ antibodies showed that $\alpha_{11}$, like $\alpha_{2\text{mt}}$, is strongly up-regulated at the protein level during differentiating cultures of human fetal muscle cells and satellite cells (Fig. 6B). To determine whether the up-regulation occurs at the mRNA level or the protein level, we analyzed $\alpha_{11}$ mRNA from different differentiation stages (day 1, day 3, and day 7) (Fig. 6C). At day 3 in differentiation medium, a strong up-regulation of $\alpha_{11}$ mRNA was already noted, establishing that the up-regulation of $\alpha_{11}$ integrin protein occurs as a result of increased transcription or mRNA stability. Based on similar SDS-PAGE migration patterns, similar behavior under reducing conditions, association with the $\beta_1$ integrin chain, and up-regulation during in vitro differentiation of human fetal myoblasts, our data show that $\alpha_{11}$ integrin is identical with $\alpha_{2\text{mt}}$.

Analysis of mRNA from the two rhabdomyosarcoma cell lines, RD and A204 (Fig. 6C), did not provide evidence for the presence of $\alpha_{11}$ in either cell line. Based on the observed up-regulation of $\alpha_{11}/\beta_1$ in human fetal muscle cells and the presence of $\alpha_{11}$ message in adult muscle, we suggest that the $\alpha_{11}$ integrin might be involved in the early steps of muscle formation and that it may fulfill a stabilizing role in adult muscle tissues. The $\alpha_7$ integrin subunit is a major $\beta_1$-associated integrin chain in muscle, but genetic deletion of $\alpha_7$ leads to a fairly mild muscular dystrophy (30). It remains to be seen whether $\alpha_{11}$ and $\alpha_7$ integrin chains have overlapping functions in muscle.

Ligand Binding Specificity of $\alpha_{11}/\beta_1$ Integrin—The I domain-containing integrins of the $\beta_1$ integrin subfamily that have been identified thus far all bind collagens (5, 15, 59). For $\alpha_1$, and $\alpha_2$ this binding capacity has been shown to reside within the I domain (17, 18). To determine whether $\alpha_{11/\beta_1}$ also binds collagen, we performed collagen type I-Sepharose chromatography of membrane proteins from surface-iodinated XXVI satellite cells. Direct analysis of the EDTA eluate revealed weak bands corresponding to the positions of $\alpha_1$, $\alpha_2$, $\alpha_{11}$, and $\beta_1$ in parallel immunoprecipitations (Fig. 7A). The EDTA eluate was concentrated by immunoprecipitation with $\beta_1$ and $\alpha_{11}$ antibodies. As shown in Fig. 7, a prominent $\alpha_{11}$ band is present in the collagen I-Sepharose eluate. The relatively weak $\beta_1$ band in the proteins captured with $\alpha_{11}$ antibodies indicates that the $\alpha_{11}/\beta_1$ heterodimer partly dissociates in the presence of EDTA.

Fig. 6. Biochemical characterization of integrin $\alpha_{11}$ chain and up-regulation of corresponding protein and mRNA in myogenic cells. A, $\alpha_{11}$ associates with the $\beta_1$ integrin chain. Human XXVI muscle cells grown in differentiation medium were metabolically labeled with $^{35}$S-cysteine/methionine, and integrins were immunoprecipitated with the indicated antibodies ($\beta_1$, $\alpha_2$, and $\alpha_{11}$). Evidence for the association of integrin $\alpha_{11}$ with the $\beta_1$-subunit was obtained by treating proteins precipitated with anti-$\beta_1$ antibodies with SDS, followed by a second precipitation with $\alpha_1$ antibodies (anti-$\alpha_{11}$ + SDS). Precipitated proteins were resolved on 7.5% SDS-PAGE gels in the absence of reducing agents, followed by fluorography. B, induction of integrin $\alpha_{11}$ upon myogenic differentiation in vitro. G6 muscle cells were metabolically labeled with $^{35}$S-cysteine/methionine when growing in proliferation medium (mb, proliferating myoblasts) and after 7 days in differentiation medium (mt, myotubes). Integrins were precipitated with antibodies to $\beta_1$ and $\alpha_{11}$, and the precipitates were resolved on 7.5% SDS-PAGE gels both under nonreducing (UNREDUCED) and reducing (REDUCED) conditions. Lanes 1, 3, 5, and 7 are immunoprecipitations with the antibody to integrin $\beta_1$, and lanes 2, 4, 6, and 8 are immunoprecipitations with the antibody to integrin $\alpha_{11}$. C, up-regulation of integrin $\alpha_{11}$ mRNA in differentiated myogenic cells. mRNA was extracted from G6 and XXVI cells growing under proliferating (p) or differentiating (d) conditions for 3 days (d3) or 7 days (d7). Total RNA was isolated from RD and A204 cells. Following separation of RNA on agarose gel and transfer to the membrane, the filter was hybridized with probes to $\alpha_{11}$ integrin ($\alpha_{11}$) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). The size of bands in RNA standard (in kb) is marked to the right.
Fig. 7. Ligand binding properties of α11 integrin. a), collagen binding integrins on XXVI cells. XXVI cells were surface-iiodinated, and integrins were analyzed by immunoprecipitation and collagen I-Sepharose affinity chromatography. Immunoprecipitation reveals the presence of β1 integrins (lane 1) α1β1 (lane 2), α111 (lane 3), and αβ5 (lane 4) at the surface of XXVI cells. EDTA-eluted proteins bound to collagen I-Sepharose contain weak bands in the position of α11, α12, and β1 integrin chains (lane 5). Immunoprecipitations with β1, α11, and αβ5 antibodies (lane 6) and α11, α1, β1, and β2 antibodies (lane 7) confirm the presence of α11, α11, and β1 in the EDTA eluate. b), α11, α1, β1, and β2, localize to focal contacts on collagen. Indirect immunofluorescent visualization of vinculin (A and B) and fibronectin (B and D). Note the localization of integrin α11 chain to focal contacts of cells allowed to attach to collagen, and its complete absence on cells seeded on fibronectin. Vinculin is found in focal contacts on both substrates. A and B show the same cell double-stained for both antigens. Scale bar, 20 μm.

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