Structure of Collagen Receptor Integrin α1I Domain Carrying the Activating Mutation E317A*§

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Matti Lahti†1, Eva Bligt51, Henri Niskanen1, Vimal Parkash9, Anna-Maria Brandt4, Johanna Jokinen1, Pekka Patrikainen5, Jarmo Käpylä5, Jyrki Heino5, and Tiina A. Salminen92

From the 1Department of Biochemistry and Food Chemistry, University of Turku, Turku FI-20014, Finland and the 9Structural Bioinformatics Laboratory, Department of Biosciences, Åbo Akademi University, Turku FI-20520, Finland

Background: The integrin αI domain undergoes a conformational change during activation.

Results: The crystal structure of an activated αI domain is different from the reported open and closed states.

Conclusion: Our structure mimics the state where the Arg287-Glu317 ion pair is just broken during the activation process.

Significance: The activation mechanism of the collagen receptor integrins differs from the other integrins.

We have analyzed the structure and function of the integrin α1I domain harboring a gain-of-function mutation E317A. To promote protein crystallization, a double variant with an additional C139S mutation was used. In cell adhesion assays, the E317A mutation promoted binding to collagen. Similarly, the double mutation C139S/E317A increased adhesion compared with C139S alone. Furthermore, soluble α1I C139S/E317A was a higher avidity collagen binder than α1I C139S, indicating that the double variant represents an activated form. The crystal structure of the activated variant of α1I was solved at 1.9 Å resolution. The E317A mutation results in the unwinding of the αC helix, but the metal ion has moved toward loop 1, instead of loop 2 in the open α1I. Furthermore, unlike in the closed αI domains, the metal ion is pentacoordinated and, thus, prepared for ligand binding. Helix 7, which has moved downward in the open α1I structure, has not changed its position in the activated α1I variant. During the integrin activation, Glu335 on helix 7 binds to the metal ion at the metal ion-dependent adhesion site (MIDAS) of the β1 subunit. Interestingly, in our cell adhesion assays E317A could activate collagen binding even after mutating Glu335. This indicates that the stabilization of helix 7 into its downward position is not required if the α1 MIDAS is already open. To conclude, the activated α1I domain represents a novel conformation of the αI domain, mimicking the structural state where the Arg287-Glu317 ion pair has just broken during the integrin activation.

Integrins are bidirectionally signaling, heterodimeric transmembrane receptors, which bind to extracellular ligands, such as matrix proteins, as well as to cytoskeletal and signaling proteins (1). Nine of 18 human integrin α subunits contain a special inserted domain (αI). This domain has a typical dinucleotide binding or Rossman fold, which closely resembles von Willebrand factor A domain (2). Therefore, the inserted domains can also be called as integrin αA domains. In the integrin αI domain a central hydrophobic β sheet is surrounded by seven amphipathic α helices. The ligand binds to a Mg2+ ion in the metal ion-dependent adhesion site (MIDAS)3 and interacts with certain surrounding amino acid residues (2).

The human αI domain integrins can be divided into two subcategories: collagen receptor and leukocyte integrins. Collagen receptor integrins (α1β1, α2β1, α10β1, and α11β1) participate in platelet adhesion, proliferation of mesenchymal stem cells, development of cartilage, and innate and acquired immunity (3). Leukocyte integrins (α4β2, α5β1, α6β4, αMβ2, and α8β2) recognize plasma proteins, e.g. component C3b in the complement system, and counterreceptors on other cells, such as intercellular cell adhesion molecules (ICAMs) (4). These integrins regulate, for example, leukocyte adhesion to endothelium and homing (5). The general structure of the αI domains in these two subcategories is similar. Collagen binding αI domain, however, contains an additional helix, called the αC helix, which is important for ligand binding (2, 6, 7).

Integrin α1β1 is a collagen receptor expressed mainly on mesenchymal cells (8). Based on the analysis of the α1-deficient mice and the in vivo experiments utilizing specific function-blocking antibodies, the biological tasks of integrin α1β1 include the regulation of immune response (9, 10), mesenchymal stem cell proliferation (11, 12), and matrix turnover (13, 14). Basement membrane collagen IV has been suggested to be the main ligand for integrin α1β1 (15, 16). Integrin α1β1 can, however, recognize many other collagen subtypes, such as fibril-forming collagens, fibril-associated collagens with interrupted triple helices, transmembrane collagens, and network-

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§1 The on-line version of this article (available at http://www.jbc.org) contains supplemental Fig. S1.

The atomic coordinates and structure factors (code 4A0Q) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

† Both authors contributed equally to this work.

1 To whom correspondence should be addressed. Tel.: 358-40-5151201; E-mail: tiina.salminen@abo.fi.

2 The abbreviations used are: MIDAS, metal ion-dependent adhesion site; ICAM, intercellular cell adhesion molecule; MEM, minimum essential medium; PDB, Protein Data Bank.
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Forming collagens (for review, see Ref. 3). Integrin α₁β₁ can also bind to distinct laminin subtypes and semaphorin 7A (17).

In α₁β₁, as in all αδIId domains, the ligand binding takes place at the MIDAS of the αδ-I domain. The atomic structures of the α₂, α₃, α₅, α₆, α₇, and δ-I domains have been solved (6, 18–23). The structure of the closed form of α₁-I and α₂-I (PDB code 1AOX) (6) are almost identical, as their structural superimposition gives a root mean square deviation of 0.62 Å for 177 Cα atoms. In the active site, the metal ion and the surrounding residues occupy identical positions in both structures, and the αC helix is stabilized by the Arg187-Glu17 (α₁-I numbering) ion pair interaction as well. The structures of the α₁-I domain in complex with ICAM-1 or ICAM-5 (24, 25), as well as α₂-I in complex with a collagenous GFOGER peptide have also been solved (26). Ligand binding opens the αδ-I domain by triggering large conformational changes in the αC helix and helix 7 and some spatial adjustments in the MIDAS. The αC helix in the α₁-I domain unwinds and swings away from the vicinity of the MIDAS. Helix 7 moves significantly downward, allowing a specific glutamate to bind to the MIDAS of the I domain of the β subunit. Glu119 in α₁, Glu129 in α₂, and Glu136 in α₂ are supposed to stabilize helix 7 in the open conformation and mediate structural changes between integrin α and β subunits (24, 27–29). These conformational modifications can also lead to the separation of the integrin leg parts and promote cell signaling.

Here, we have characterized the collagen binding properties of an activated variant of the α₁-I domain, harboring a gain-of-function mutation E317A (30). To promote protein yield, solubility, and crystallization, a double variant with an additional C139S mutation was used. In cell adhesion assays E317A mutation promoted binding to collagen. Similarly, double mutation C139S/E317A increased adhesion compared with C139S alone. Furthermore, E317A could increase cell adhesion even after the connection between α₁-I and β subunits had been prevented by E335A mutation. The solved 1.9 Å crystal structure of the activated variant of α₁-I has a novel conformation, which is different from the previously reported “open” and “closed” αδ-I domains.

EXPERIMENTAL PROCEDURES

Materials—Rat collagen I was purchased from Sigma-Aldrich, and collagen IV from mouse Engelbreth-Holm-Swarm tumor was from BD Biosciences. Bovine serum albumin (BSA) was from Bovogen Biologicals.

Mutagenesis of Human Integrin α₁ and Its α₁-I Domain—The α₁-I domain including the amino acids (138-ECS-LEATA-338) was previously cloned into the pGEX-4T vector (Amersham Biosciences) (23, 31). The full-length α₁ human integrin constructed in pcDNA3.1/Z-2 plasmid (Invitrogen) was a kind gift from Dr. Pauli Ollikka (Biotie Therapies Corp.). The point mutations (C139S, E317A, C139S/E317A, E335A, and E335A/E317A) to both full-length α₁ and α₂-I were carried out using a QuikChange site-directed mutagenesis kit (Stratagene). All clones were sequenced to ensure that undesired mutations were not introduced during PCR. Plasmid constructs (pcDNA3.1/Z-2/α₁ and its variants) were transfected into Chinese hamster ovary cells (CHO) cells (American Type Culture Collection, ATCC) as described previously for α₂ (29). FuGENE6 (Roche Applied Science) was used for transfection in CHO cells. Two days after transfection, transformants were selected with 150 μg/ml Zeocin (Invitrogen) in α-MEM. After 2 weeks, positive cells were stained with integrin α₁ antibody (purified anti-human CD49a, SR-84; BD Pharmingen) and anti-mouse IgG FITC antibody (Santa Cruz Biotechnology) and collected as mixed populations using FACS Vantage SE flow cytometry (BD Biosciences).

Cell Adhesion Assays—Attachment and spreading of chimeric α₁β₁ (human α₁, hamster β₁) expressing CHO cells was tested with xCELLigence real-time cell analyzer (RTCA; Roche Applied Science). This technology measures impedance at the bottom of a microtiter plate well and allows estimating the progression of cell attachment and spreading. E-plates 96 (Roche Applied Science) were coated either with collagens I, IV (5 μg/cm²/well; 16.4 μg/ml) or BSA (0.1%) in Dulbecco’s phosphate-buffered saline (PBS; Sigma) overnight at 4 °C. Before coating collagen I was diluted in acetic acid (0.1 M) to keep it in monomeric, triple helical form. Coated wells were washed with PBS and blocked with BSA for 1 h (0.1% in PBS, 5% CO₂, 37 °C). After blocking, BSA was removed, and α-MEM (without FCS) was added to the wells. The background signal was measured, and 40,000 cells/well were added. BSA was used as the negative control, and its signal was measured for each cell line with three parallel wells (data not shown). The Mann-Whitney U test with SPSS software (version 16.0; IBM) was used for statistical analysis of the data collected at the 1-h time point. Cell adhesion was followed for 2 h at 37 °C (5% CO₂). The cells used in these experiments were collected from almost confluent culture plates. Trypsin-EDTA solution (Sigma) was used to remove the cells from the plate, and trypsin inhibitor (Sigma) was added. The cells were spun down at 500 × g for 5 min at 37 °C, and the pellet was resuspended using α-MEM medium without FCS.

Protein Expression and Purification—For solid phase binding assays, protein expression and purification were performed as described earlier (30). Briefly, glutathione S-transferase (GST) fusion proteins were expressed in Escherichia coli BL21 Tuner™ (Novagen). For crystallization of α₁-I C139S/E317A, the protein was produced using 100 ml of overnight culture to inoculate 15 liters of LBAMPMedium and cultured in Bioengineering fermentor (Bioengineering AG) at 37 °C until the A₆₀₀ nm reached 0.5–0.6. To induce expression of the protein, isopropyl β-D-1-thiogalactopyranoside was added to a final concentration of 0.4 mM. Protein production was continued at room temperature for 6 h and at 10 °C overnight. The cells were harvested by centrifugation, and the GST fusion protein was purified as described earlier (30). GST was removed by thrombin digestion (GE Healthcare). 100 units of thrombin were added every 2nd h for 8 h. GST was separated from the α₁-I domain by GST-affinity chromatography using disposable columns (Bio-Rad). Protein was further purified by gel filtration chromatography (HiLoad™26/60 Superdex™200 preparation grade; GE Healthcare) using Äkta FPLC. The purified protein was concentrated using Centriprep centrifugal filter device (Millipore) followed by buffer exchange to 40 mM Tris (hydroxymethyl) aminomethane, pH 7.2, 2 mM MgCl₂, and 20% glycerol. Finally, the protein was concentrated using Centricon centrifugal filter device (Millipore) to 23–28 mg/ml. The purity of the protein...
was checked by electrophoresis on 8–25% gradient polyacrylamide gels in the presence of 0.55% SDS using the Phast System (Amersham Biosciences).

**Solid Phase Binding Assays**—Binding assays were carried out as described previously (30, 32). 96-well plates were coated overnight at 4 °C with either collagens I, IV (5 μg/cm²/well) or BSA (3.75%) that was used for blocking the wells. GST fusion α1 I domains were allowed to bind for 1 h in the presence of 2 mM MgCl2 in Delfia® assay buffer (PerkinElmer Life Sciences). Wells were washed three times, and the signal was detected with Delfia® europium-labeled anti-GST antibody (PerkinElmer Life Sciences). Label was dissociated with Delfia® enhancement solution (PerkinElmer Life Sciences), and the signal was determined using a time-resolved fluorescence spectrophotometer (Victor3 multilabel counter; PerkinElmer Life Sciences). Estimates for the dissociation constants (Kd) were obtained using the following equation: measured binding = maximal binding/(1 + Kd/[I]).

**Crystallization and Data Collection**—Initial screening for crystallization conditions was done with the sparse matrix screen JCSG+ Suite (Qiagen) using the hanging drop vapor diffusion method. The crystals were grown at 8 °C by mixing 1 μl of 26 mg/ml protein solution in 40 mM Tris, pH 7.4, 2 mM MgCl2, and 20% glycerol with 1 μl of the well solution, which contained 1.6 mM trisodium citrate. In a week, the crystals grew to the final size of 0.3 × 0.1 × 0.1 mm³. The crystal was picked directly from the crystallization plate and flash frozen with liquid nitrogen. The crystal diffracted to 1.9 Å, and the data were collected on ADSC Quantum Q210 detector installed on beamline ID 14-1 at the European Synchrotron Radiation Facility (ESRF, France). The data were integrated and scaled in space group P3 using the XDS and XSSCALE programs (33).

### Table 1

**Statistics of the structure determination of the activated α1 I C139S/E317A**

| Data collection |  |
|-----------------|-----------------|
| Beamsline       | ID14-1 (ESRF, Grenoble) |
| Space group     | P3 |
| Unit cell dimensions (Å, °) | a = b = 95.5, c = 37.8; α = β = 90, γ = 120 |
| Resolution limits (Å) | 47.86–1.90 (2.05–1.90) |
| No. of unique reflections | 172,075 (34,565) |
| Wavelength (Å) | 0.93 |
| Matthews coefficient (Å³/Da) | 2.26 |
| Solvent content (%) | 45.5 |
| Molecules/asymmetric unit | 2 |
| Completeness (%) | 99.3 (99.2) |
| Redundancy | 5.7 (5.7) |
| Rmerge (%) | 6.1 (45.6) |
| Rmerge-F (%) | 6.7 (50.2) |
| Average I/σ | 8.7 (41.9) |
| Wilson B-factor | 20.1 (4.1) |
| Wilson B-factor | 23.6 |

**Refinement statistics**

| PDB code | 4A0Q |
| Resolution range (Å) | 20.00–1.90 |
| Reflections | 28,552 |
| Rmerge (%) | 18.6 |
| Rmerge (%) | 22.9 |
| No. of amino acids | 374 |
| No. of water molecules | 185 |
| Average B-factor for all atoms (Å²) | 28.3 |
| Ramachandran plot (%) | Most favored 97.5 |
| Additional allowed | 2.2 |
| Disallowed regions | 0.3 |

**Structure Determination, Model Building, and Refinement**—The solvent content of the crystal was 45.5%, with two chains in the asymmetric unit (Table 1). The structure of α1 I C139S/E317A was determined by molecular replacement using the program Molrep (34, 35). We searched for two α1 I domains using closed α1 I (PDB code 1PT6) (23) as a search model. 5% of the reflections (1,428) were randomly selected for Rfree calculation, and the remaining data (28,552) were used in refinement (Table 1). Using Refmac5 (36), the initial R-factor after rigid body refinement was 27.6% (Rfree = 26.6). ARP/wARP Solvent (37) was used for the addition of water molecules. The Refmac5 refinement was followed by manual model building in COOT (38). Iterative model building and refinement gave a final model with R-factor 18.6% (Rfree = 22.9). The electron density quality was good throughout both chains except for the weak electron density of the residues 286–291 in chain B and the total lack of electron density for the residues 286–290 in chain A. The final model was validated using Molprobity (39). All the structural figures were made with PyMOL (40).

### RESULTS

**Activation of Integrin α1β1 by α1 E317A Mutation Leads to High Avidity Cell Adhesion to Collagens I and IV**—In our previous studies, we have identified α1 E317A as a gain-of-function mutation, which improves collagen binding considerably (30). To investigate the integrin activation further, we used CHO cells transfected to express wild-type (WT) α1 and mutant integrins (α1, E317A, α1, C139S, α1, C139S/E317A). Flow cytometry experiments confirmed comparable expression levels for the variant and WT α1 integrins (supplemental Fig. S1). The effect of integrin mutations on cell adhesion was analyzed using the xCELLigence instrument. This technology measures
impedance at the bottom of a microtiter plate well and allows following the progression of cell attachment and spreading. The E317A mutation in α1 caused significant increase in cell adhesion to both collagen I and IV compared with WT α1 (p < 0.002, Mann-Whitney U test, cell adhesion to collagen I or collagen IV at 1-h time point). Similarly, cell adhesion studies with α1 C139S/E317A indicated that the double variant is a better binder of collagens than α1 C139S (p < 0.002, Mann-Whitney U test, cell adhesion to collagen I or collagen IV at 1-h time point) (Fig. 1). Even though C139S alone reduces adhesion, E317A mutation could significantly activate cell adhesion even in the presence of C139S. Importantly, WT and variant α1 integrins showed better adhesion to collagen IV compared with collagen I, suggesting that the mutations had not changed the ligand specificity.

**As a Soluble, Recombinant Protein the C139S/E317A Variant of the α1 Domain Shows an Increased Avidity to Collagen Compared with the α1 C139S—**The collagen binding properties of the soluble, recombinant α1 C139S and α1 C139S/E317A variants were studied in a solid phase binding assay. The double variant containing the activating E317A mutation (α1 C139S/E317A) bound to both collagen I and IV significantly better than α1 C139S (Fig. 2). Furthermore, based on Kd values, α1 C139S binds to collagens I and IV with similar avidity, whereas α1 C139S/E317A binds to collagen IV more tightly than to collagen I. The results suggest that E317A activates the α1 domain even in the presence of the C139S mutation. Because of its higher avidity, we have named α1 C139S/E317A as an activated form of α1.

**X-ray Structure of Activated α1 C139S/E317A—**The structure of the double variant C139S/E317A of the ligand-free α1 domain was solved by molecular replacement using the closed conformation of α1 (PDB code 1PT6) (23) as a search model. We refined the structure using Refmac5 (36) to a final R-factor of 18.6% (Rfree = 22.9%). Statistics for the data processing and structural refinement are summarized in Table 1. The asymmetric unit in the crystal contains two molecules, which are identical, and their structural superimposition gives a root mean square deviation of 0.06 Å for 177 Cα atoms. Each chain comprises residues 142–333, 185 water molecules, and a Mg2+ bound at the MIDAS. The region of residues 138–141 in the N terminus of both chains is disordered, so the mutation C139S is not visible in the structure. The electron density map was good throughout the structure except for the loop region 286–290 (Fig. 3). The residues 286–290 in the loop region in chain A could not be built because of the lack of electron density, and only the main chain atoms are built for chain B (Fig. 3B). In the following, we therefore discuss only chain B. The core structure is similar to the closed form of the WT α1 with a central hydrophobic β sheet sandwiched by six amphipathic α helices.

**Activating E317A Mutation Caused Significant Conformational Changes in αC Helix—**The αC helix (residues 283–287) stabilized by the Arg287–Glu317 ion pair interaction plays important role in collagen binding (7). The αC helix unwind...
when collagen binding to the MIDAS disrupts Arg\textsuperscript{287}-Glu\textsuperscript{317} interaction (26). Compared with the closed form of α\textsubscript{1}L (Fig. 4A, green), where Tyr\textsuperscript{285} restricts the ligand binding to the MIDAS, in our activated C139S/E317A structure (Fig. 4A, salmon) the αC helix unwinds and Tyr\textsuperscript{285} is moved away from the MIDAS. As a result of this movement, the active site opens. This is to our knowledge the first structure of the α\textsubscript{1}L domain with the unwound αC helix in the absence of collagen.

**MIDAS of Activated α\textsubscript{1}L**—In the MIDAS of the integrin α\textsubscript{1}L domain residues from three loops (L1, L2, and L3) surround the metal ion in the active site and coordinate the metal directly or indirectly (Fig. 5). Compared with the hexacoordination of the metal in previously published α\textsubscript{1}L and α\textsubscript{1}I structures, the metal ion in our activated α\textsubscript{1}L structure forms a pentacoordinated complex (see below), where Ser\textsuperscript{152} and Ser\textsuperscript{154} from L1 and three water molecules coordinate the metal through their hydroxyl oxygen (Fig. 5B).

**Activated α\textsubscript{1}L C139S/E317A Shows Differences in MIDAS, αC Helix, Helix 6, and Helix 7 Compared with Open α\textsubscript{1}I Structure**—The core structure of our activated α\textsubscript{1}L is very similar to the open α\textsubscript{1}I. However, it is different from the open α\textsubscript{1}I with respect to the MIDAS, the αC helix, helix 6, and helix 7. The metal coordination in our activated α\textsubscript{1}L structure is slightly different from both closed and open α\textsubscript{1}I (Fig. 5, A–C). The direct binding of Ser\textsuperscript{152} and Ser\textsuperscript{154} (α\textsubscript{1}I numbering) to the metal is conserved in all the structures. In the activated α\textsubscript{1}L, the metal moves (2.2 Å) toward L1 with respect to its position in the closed α\textsubscript{1}I (Fig. 5B). This is similar but not identical to the metal ion movement between the open and closed α\textsubscript{1}L, where the metal moves 2.6 Å toward L2 (26). Because of the metal ion movement toward L1 in the activated α\textsubscript{1}L, the conserved threonine (Thr\textsuperscript{221}) on L2 interacts with the metal via another water molecule (Fig. 5B) unlike in the open α\textsubscript{1}L where Thr\textsuperscript{221} binds directly to the metal (Fig. 5C). Similar to the open α\textsubscript{1}I structure, Asp\textsuperscript{284} in the activated α\textsubscript{1}L makes a water-mediated contact to the metal (Fig. 5E).

As in the open α\textsubscript{1}L structure, the αC helix of the activated double variant has unwound to a loop structure. Although the residues near the MIDAS are mostly conserved, the residues Ser\textsuperscript{284} and Tyr\textsuperscript{285} in the αC helix of α\textsubscript{1}L correspond to Tyr\textsuperscript{285} and Leu\textsuperscript{386} in α\textsubscript{1}I, respectively (Fig. 5D). Despite these differences in the amino acid sequences, the tyrosine residue is turned away from the MIDAS in both open α\textsubscript{1}I and activated...
Activating Mutation of Integrin $\alpha_I$ Domain

FIGURE 4. Superposition of the activated and the closed forms of the $\alpha_I$ domain. A, overall view of the $\alpha_I$ C139S/E317A superimposed on the closed form of the integrin $\alpha_I$ domain. The closed form is shown in salmon, and the activated conformation is in green. The Mg$^{2+}$ ions are shown as spheres in respective colors. The $\alpha_C$ helix is unwound to a flexible loop in the activated structure. The region of the loop, residues 286–290, is presented in gray. The position of helix 7 is identical in both structures. B, close-up view of the MIDAS. Tyr$^{285}$ points away from the MIDAS in the activated structure whereas it covers the MIDAS in the closed form. In the activated $\alpha_I$ structure, the position of the Mg$^{2+}$ ion has shifted by 2.2 Å. C, E317A mutation breaking the ion pair Arg$^{287}$-Glu$^{317}$ in the activated $\alpha_I$ structure.

FIGURE 5. Comparison of the closed, activated, and open form of $\alpha_I$ structures. A, $\alpha_I$ in the closed conformation (salmon, PDB code 1PT6). B, activated conformation of $\alpha_I$ C139S/E317A (green). C, open $\alpha_I$ (yellow, PDB code 1DZI). The brown sphere represents Glu$^{11}$ from the bound ligand. The extra turn on top of helix 6 resulting from the unwinding of the $\alpha_C$ helix is shown in cyan. D, residues corresponding to Ser$^{284}$ and Tyr$^{285}$ in $\alpha_I$ (salmon, panel A) are Tyr$^{285}$ and Leu$^{286}$ in $\alpha_I$ (yellow, panel C). E, close-up view of the comparison of the MIDAS of the three structures. F, side view of the open $\alpha_I$ (yellow) and the activated $\alpha_I$ C139S/E317A (green) to see differences in helix 7. The arrow points to the difference in the loop structures before helix 7.
Activating Mutation of Integrin \(\alpha_1\) Domain

The mutation E318A in \(\alpha_1\) activates collagen and laminin binding (30, 41). We have previously introduced the equivalent E317A mutation to \(\alpha_1\) and shown its activating character (30). To get the first three-dimensional structure of an activated collagen receptor \(\alpha_1\) without a ligand, we had to introduce an additional mutation, namely C139S, to increase the protein yield and solubility. Equivalent cysteine to serine mutation has been used to decrease the aggregation of \(\alpha_1\) domain (42). We used both solid phase binding assay and cell adhesion assay to confirm that the mutant \(\alpha_1\) C139S/E317A still represents an activated \(\alpha_1\) domain. Kinetically, \(\alpha_1\) C139S/E317A resembles \(\alpha_1\) WT, binding more tightly to collagen IV than to collagen I. Interestingly, C139S mutation alone decreased both the \(\alpha_1\) domain binding to collagen and cell adhesion. We also know based on our earlier experiments that the short \(\alpha_1\) and \(\alpha_1\) domain constructs lacking this particular cysteine show weaker binding to collagens. In the \(\alpha_1\) heterodimer (PDB code 3K6S) (43), the corresponding cysteine links the \(\alpha_1\) domain to the \(\beta\) propeller domain in the \(\alpha_\chi\) subunit by forming a disulfide bond with a cysteine residue in the \(\beta\) propeller. The loss of this stabilizing disulfide would explain the decreased activity of the \(\alpha_1\) heterodimer harboring the \(\alpha_1\) C139S mutation. However, the stabilization mechanism of C139 in the recombinant \(\alpha_1\) domain remains unknown.

Collagen binding to the \(\alpha_1\) domain initiates the loss of the Arg287-Glu317 ion pair interaction (Fig. 4C), which leads to the unwinding of the \(\alpha\) helix. We originally predicted that the loss of the salt bridge in the \(\alpha_1\) E317A variant would trigger large conformational changes similar to those seen in the collagen-like peptide bound \(\alpha_1\) (26), including the downward movement of helix 7 (30). However, our activated mutant structure revealed unexpected and interesting mechanism for the \(\alpha_1\) domain activation without any change in the position of helix 7. Even though the activation of \(\alpha_1\) domain is due to the gain-of-function mutation and, therefore, not physiological, the unveiled atomic structure can be used to explain the activation mechanism.

Enhanced ligand binding of the \(\alpha_1\) C139S/E317A variant could be explained by the fact that Tyr285, which covers the MIDAS in the closed form (23), has moved aside and unwinds the \(\alpha\) helix making the MIDAS more accessible for the ligand. Tyr285 in \(\alpha_1\) is not conserved and does not correspond to Tyr285 in \(\alpha_1\) (Fig. 5D); however, it seems to take the role of Tyr285 in \(\alpha_1\) (Fig. 5F). This could result in differences between the open forms of \(\alpha_1\) and \(\alpha_1\), because the \(\alpha\) helix might not be long enough in \(\alpha_1\) to reorganize an additional turn on helix 6. This would also mean that the open form of \(\alpha_1\) is different from the homology model of the open \(\alpha_1\) (23, 30), which was predicted based on the ligand-bound \(\alpha_1\) structure (26). It seems reasonable to speculate that the change in the metal ion coordination (from pentacoordinated to hexacoordinated) upon ligand binding to the activated mutant \(\alpha_1\) would induce the change of the activated form to a fully open form of \(\alpha_1\) where helix 7 is in the downward position. Because the opening of the \(\alpha\) helix does not have an effect on helix 7, we propose that interaction with collagen or a collagen like peptide is necessary to push helix 7 into the position required for interaction with the MIDAS of the \(\beta_1\) domain. Recently, the structure of the \(\alpha_1\) domain in complex with its ligand ICAM-5 showed the unusual allosteric mobility of helix 7 (25).

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activin-based plasminogen activator receptor (58). There seem to be direct or indirect interactions between integrins and these proteins, but very little is known about the structural basis of the mechanism that they use in integrin regulation. It is possible to speculate that the integrin conformation can be modified by other membrane proteins, which would e.g. induce the breakage of the Arg287-Glu317 salt bridge and consequently unwind the c-helix. It remains to be seen, however, whether the novel α1I domain conformation represents this kind of activation mechanism.

To conclude, we have solved to our knowledge the first atomic structure of an activated collagen receptor α1I domain. The conformational changes explain the enhanced ligand binding, but are clearly different compared with the previously published closed/intermediate/open α1 domain structures. Our data indicate that the activated α1I domain may also differ from the ligand-bound, open α1I domain.

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