We have determined the high resolution crystal structure of the I domain from the α2β1, a cell surface adhesion receptor for collagen and the human pathogen echovirus-1. The domain, as expected, adopts the dinucleotide-binding fold, and contains a metal ion-dependent adhesion site motif with bound Mg$^{2+}$ at the top of the β-sheet. Comparison with the crystal structures of the leukocyte integrin I domains reveals a new helix (the C-helix) protruding from the metal ion-dependent adhesion site face of the domain which creates a groove centered on the magnesium ion. Modeling of a collagen triple helix into the groove suggests that a glutamic acid side chain from collagen can coordinate the metal ion, and that the C-helix insert is a major determinant of binding specificity. The binding site for echovirus-1 maps to a distinct surface of the α2I domain (one edge of the β-sheet), consistent with data showing that virus and collagen binding occur by different mechanisms. Comparison with the homologous von Willebrand factor A3 domain, which also binds collagen, suggests that the two domains bind collagen in different ways.

The integrins are a family of plasma membrane proteins that transduce bidirectional signals between the cytoplasm and the extracellular matrix or other cells (1). The integrin α2β1 is expressed on a variety of cell types, serving as the collagen receptor on platelets and fibroblasts, and as both a collagen and laminin receptor on endothelial and epithelial cells (2, 3). It also acts as the receptor for the human pathogen echovirus-1 (4). In common with six other integrin αβ pairs, α2β1 binds collagen with a central parallel β-sheet surrounded on both sides by α-helices (13). Apart from the highly conserved residues that directly coordinate the metal, the upper surface of the domain surrounding the MIDAS motif is highly variable, suggesting that this metal-dependent site is essential for the interaction of αLβ2 with intercellular adhesion molecule-1 that are located on the MIDAS face surrounding the site of metal coordination. In addition, two of the epitopes for function-blocking antibodies map to the same face (19). Similarly, Rieu et al. (20) showed that residues essential for the binding of the hookworm pathogen, neurtophil inhibitory factor, a protein that blocks the binding of natural ligands to αMβ2, cluster around the MIDAS face of αI.

Crystal structures have previously been reported for the αMβ2 and αM-I domains with bound Mg$^{2+}$ and Mn$^{2+}$ (13, 21–23). The crystal structure of an A-domain from von Willebrand Factor (vWF-A3) has also been solved recently (24). We now report the crystal structures of the α2-I domain, which we determined as a first step in understanding the atomic level determinants of collagen binding and compare the structure with other A/I domains. vWF-A3 also binds collagen, suggesting that the two domains might have similar binding motifs. Our crystal structure suggests that they do not.

**EXPERIMENTAL PROCEDURES**

Purification and Crystallization of the α2-I Domain—Human α2-I domain (residues 140–337) was expressed as a glutathione S-transferase fusion protein in *Escherichia coli*, cleaved, and purified as described previously (25). The protein was next loaded onto an affinity iminodiacetic acid-Sepharose column (Pharmacia) charged with Ni$^{2+}$ and

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The first crystal structure of an integrin I domain, from αMβ2, showed that it adopts the dinucleotide-binding fold, with a central parallel β-sheet surrounded on both sides by α-helices (13). In this class of fold, a functional surface of the domain always lies at the C-terminal end of the β-sheet (14). In the I domain, a novel cation coordination sphere is located there, and in the αM-I domain crystal structure with bound Mg$^{2+}$, a glutamate side chain from a neighboring I domain in the crystal lattice completes the octahedral coordination sphere of the metal. This led to the suggestion that the glutamate behaves as a ligand mimic, as most integrin ligands possess a critical aspartate residue (or glutamate) as a key feature of their integrin-binding motifs, and mutation of any of the metal-coordinating side chains of the I domain (8, 15–17) abolishes binding in a dominant negative fashion. This motif was therefore dubbed the metal-ion-dependent adhesion site (MIDAS) (13). Apart from the highly conserved residues that directly coordinate the metal, the upper surface of the domain surrounding the MIDAS motif is highly variable, suggesting that the metal-Glu/Asp bond contributes some but not all of the binding energy, with the rest of the energy, and the specificity, arising from further interactions (ionic/polar/hydrophobic) between complementary surfaces of the integrin and ligand. In support of this notion, Huang and Springer (18) utilized mouse chimeras and site-specific mutagenesis to demonstrate that residues essential for the interaction of αLβ2 with intercellular adhesion molecule-1 are located on the MIDAS face surrounding the site of metal coordination. In addition, two of the epitopes for function-blocking antibodies map to the same face (19). Similarly, Rieu et al. (20) showed that residues essential for the binding of the hookworm pathogen, neutrophil inhibitory factor, a protein that blocks the binding of natural ligands to αMβ2, cluster around the MIDAS face of αI.

Crystal structures have previously been reported for the αL and αM-I domains with bound Mg$^{2+}$ and Mn$^{2+}$ (13, 21–23). The crystal structure of an αI domain from von Willebrand Factor (vWF-A3) has also been solved recently (24). We now report the crystal structure of the α2-I domain, which we determined as a first step in understanding the atomic level determinants of collagen binding and compare the structure with other A/I domains. vWF-A3 also binds collagen, suggesting that the two domains might have similar binding motifs. Our crystal structure suggests that they do not.

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† The abbreviations used are: MIDAS, metal ion-dependent adhesion site; vWF-A3, von Willebrand Factor A3 domain; αMβ2, α-I domain with bound Mn$^{2+}$; αMβ2-I, α-M-I domain with bound Mn$^{2+}$; αMβ2-I, α-M-I domain with bound Mg$^{2+}$; RMS, root mean square.

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eluted with a linear gradient of 0–200 mM imidazole. Fractions were pooled, diluted 3-fold with distilled water and concentrated to 10 mg/ml.

Crystals grow to a typical size of 0.5 mm in 6–8 days and belong to the space group P1 with cell dimensions: \( a \times b \times c = 88.3 \times 43.3 \times 43.3 \) Å, \( \alpha \times \beta \times \gamma = 106.6^\circ, 110.6^\circ, 107.6^\circ \). Isomorphous crystals grow in the presence of manganese.

Data Collection and Structure Determination—A 2.5 Å data set (see Table I) was collected from a single crystal mounted and frozen in a cryostat at 100 K using a Rigaku RU-200 x-ray generator with focusing mirrors and an RAXIS II image plate. Data were reduced with DENZO and scaled with SCALEPACK (26) with an \( R_{merge} = 4.2\% \), and 95% completeness to 2.5 Å resolution. This data set was used to perform the molecular replacement calculations and the early stages of refinement. A room temperature data set was subsequently collected from an imperfect twin. As the twins of the crystal were randomly oriented and diffracted with approximately equal intensity, both triclinic lattices could be indexed and data merged, with an \( R_{merge} = 11.2\% \) (31% in the outer shell) and completeness of 96.5% to 1.9 Å resolution. A 7 Å peak on the x = 180° section of a self-rotation function calculated using GLRF (27) indicated the presence of two molecules in the asymmetric unit, consistent with a solvent content of 55%.

Molecular replacement was performed with AMoRe (28), using the superposed structures of \( 55\% \) of the molecules in the asymmetric unit, consistent with a solvent content of 55%.

The model includes residues 139–339 (including several side chains and the C-terminal helix like peptide (Pro-Hyp-Gly)\(_4\)-Pro-Hyp-Ala-(Pro-Hyp-Gly)\(_5\)) of the preferred \( \alpha \) helix(+) conformer of collagen. The collagen model and I domain were then manually docked as rigid bodies by constraining the distance between the glutamate carboxylate and the metal ion to 2 Å (the observed bond distance in the oM-I domain) and by minimizing intermolecular steric clashes (monitored using the CCP4 program CONTACT (28)). With Glu at either the X or Y position, a convincing fit could be found with only minor steric clashes. These clashes could be relieved either by trimming 3 or 4 of the collagen Pro/Hyp side chains to Ala or Gly, or by allowing a small amount of alternate side chain conformations on the I domain to adopt alternate rotamer conformations. When the Glu was replaced by an Asp, severe steric clashes occurred between backbone atoms of the two docking partners.

### RESULTS AND DISCUSSION

**Crystal Structure of the a2-I Domain**—The structure, as expected, adopts the classic dinucleotide-binding fold, with seven helices surrounding a core of five parallel \( \beta \)-strands and one short antiparallel \( \beta \)-strand (Figs. 1 and 2). Compared with the \( \alpha \)M and \( \alpha \)L-I domains, the most striking difference is a new turn-and-a-half of \( \alpha \)-helix, residues 284–288, which we call the C-helix, extending from the top of strand \( \beta \)E and protruding from the MIDAS face (see below). There is a buried glutamic acid in place of the usual glycine in the position following the MIDAS aspartic acid (DESNs), but this is accommodated without distortion of the MIDAS motif. Space is created by a 1-residue insertion in the \( \alpha \)3-\( \alpha \)4 loop that wraps around the top of \( \beta \)A and \( \beta \)B and the charge is neutralized by a salt bridge to Arg\(^{184}\) from \( \beta \)C. A buried water molecule adjacent to the MIDAS motif is closely conserved in \( \alpha \)M-I and \( \alpha \)L-I; it makes hydrogen bonds to the main chain carbon oxygen of Gly\(^{285}\) and Thr\(^{253}\) and to the carboxylate of Glu\(^{289}\).

The buried Phe in \( \alpha \)M-I and \( \alpha \)L-I (Ile in vWF-A3) at the top of helix \( \alpha \)7 (which becomes exposed in the "active" conformer of \( \alpha \)M-I (21)) is replaced by a glutamic acid in the a2-I domain (Glu\(^{118}\)). The side chain turns upwards to avoid complete burial, creating a cavity which is filled by a water molecule that is not found in \( \alpha \)L-I, \( \alpha \)M-I, or vWF-A3. The water molecule hydrogen bonds to the carboxylate of the MIDAS aspartate (Asp\(^{254}\)), the main chain nitrogens of Gly\(^{284}\) and Tyr\(^{255}\) as well as the carboxylate of Glu\(^{289}\). A salt bridge is provided by the side chain of Arg\(^{288}\) from the C-helix. The orientation of the \( \alpha \)7 helix is nevertheless very similar to that in the \( \alpha \)M-I\( ^{44s}\) structure.

Sequences of a2B1 have been reported from human, cow, mouse, and pig. Within the I domains, 43 positions are not invariant. All these lie on the surface of the molecule, except for two conservative changes in the hydrophobic core (Val\(^{182}\) and Met and Leu\(^{328}\) in bovine). The only change found is on the MIDAS face, which is not conserved in any of the \( \alpha \)M-I\( ^{44s}\) structures.

Although the two molecules in the crystal unit cell were refined independently, the overall structures are almost identical (RMSD = 0.25 Å for main chain atoms) except for the chain termini. Both chain termini are ordered to a greater extent than in other I domain structures. The N terminus extends 5 residues before strand \( \beta \)A, and the C terminus 3 residues before strand \( \beta \)A, and the C terminus 3 residues before strand \( \beta \)A.
residues beyond helix $\alpha 7$. In the crystal, a disulfide bridge is formed between the N-terminal cysteine residues (Cys$^{140}$) of the two molecules in the asymmetric unit, and this results in different conformations of the termini. It is unlikely that such a disulfide bond forms in vivo, and Springer (31) has predicted that Cys$^{140}$ makes a disulfide bridge with a cysteine residue (residues 244–246). The side chain of Ile$^{335}$ packs into this crevice formed by the N-terminal loop (residues 138–142) and the C-terminal residues (335–337) pack into a crevice formed by the 4-1 loop of his propeller model. In one molecule, the C-terminal residues (335–337) pack into a crevice formed by the N-terminal loop (residues 138–142) and the $\alpha 4$-$\beta$D loop (residues 244–246). The side chain of Ile$^{335}$ packs into this crevice, the main chain makes a number of $\beta$-sheet hydrogen bonds to both loops, and the N and C termini are brought into very close proximity. In the second molecule in the crystal, it appears that disulfide bond formation induces a new conformation in the N-terminal loop which squeezes the C-terminal residues out of the crevice, and we suggest that the first molecule better reflects the native conformation of the domain termini in the intact integrin.

**The MIDAS Motif**—The MIDAS motif (Fig. 3) binds a magnesium ion in the $\alpha 2$-I domain, as expected given the strict sequence conservation of the motif throughout the integrin I domains (the MIDAS motif in the vWF-A3 domain is not strictly conserved, and the vestigial motif does not bind a metal ion (24)). The metal is directly coordinated by three side chains (from residues Ser$^{153}$, Ser$^{155}$, and Asp$^{254}$) and three water molecules, making strong bonds (2.0 ± 0.1 Å) in an octahedral arrangement. Asp$^{151}$ makes hydrogen bonds to Ser$^{153}$ and a water molecule (2.7–2.9 Å) but no direct bond. Thr$^{221}$ does not coordinate the metal directly (Mg-OH(Thr) = 4.1 Å), but makes a hydrogen bond (2.9 Å) to one of the water molecules. This coordination is very similar to that found in the Mn$^{2+}$-bound structures of $\alpha L$-I and $\alpha M$-I ($\alpha L^{Mn}$-I and $\alpha M^{Mn}$-I, defined as the “inactive” form by Lee et al. (21)), but different from the coordination observed in the $\alpha M$-I structure with bound Mg$^{2+}$ ($\alpha M^{Mg}$-I, the active form). It is the first high resolution crystal structure of the inactive conformer with bound Mg$^{2+}$, and confirms the coordination predicted from the lower resolution (2.8 Å) structure of $\alpha L$-I with bound Mg$^{2+}$ (23). The role of the MIDAS threonine is intriguing; it is the only MIDAS residue that is absolutely critical for collagen binding to the recombinant $\alpha 2$-I domain (32), and it is also critical for ligand binding in the $\alpha M$-I and $\alpha L$-I domains (33). Only in the active $\alpha M^{Mg}$-I domain does the threonine coordinate the metal directly, suggesting that the threonine is required for stability of the active conformer and supporting the theory of tertiary structure change within the I domain (21). It appears that using modified protein, the requirement for cation can be circumvented (see below), but it is puzzling that the threonine remains essential under those conditions also (32). This issue will only be properly resolved by structure determination of an authentic I domain-ligand complex.

**Comparison with Other A/I Domains**—The central core of five parallel $\beta$-strands and one short antiparallel $\beta$-strand is highly conserved among the integrin I domains and vWF-A3, with RMS deviations of 0.6–0.7 Å (Figs. 1 and 2). The $\beta$-BC hairpin and a $\beta$-bulge at the end of $\beta C$ are almost identical in all four structures. By contrast, the helices are more variable, with only helices $\alpha 1$ and $\alpha 4$ showing a general agreement of length and orientation. Helix $\alpha 2$ is replaced by a short turn and helix $\alpha 3$ extended by a turn in $\alpha 2$-I, and vWF-A3. The C-terminal helix, $\alpha 7$, has a similar conformation in $\alpha M^{Pn}$-I, $\alpha 2$-I, and vWF-A3, but is different in $\alpha L$-I, where the helix splays out from the side of the domain, exposing a large hydrophobic crevice that is filled by a hydrophobic C-terminal sequence from another molecule in the crystal lattice (22). The conformation of helix $\alpha 7$ is also very different in the active conformer of $\alpha M^{Pn}$, where a functional role in propagating structural changes from the MIDAS face to the rest of the integrin has been proposed (21).

**The MIDAS Face**—The loops surrounding the MIDAS motif, which comprise the MIDAS face, are $\beta A$-$\alpha 1$, $\alpha 3$-$\alpha 4$, $\beta D$-$\alpha 5$, and $\beta E$-$\alpha 6$ (Fig. 4). These loops, except for $\beta E$-$\alpha 6$, have been implicated in ligand binding to the $\alpha L$-I and $\alpha M$-I domains by mutagenesis experiments (18–20). The loops have highly variable surface-exposed residues in all of the A/I domains, even when the main chain conformation is conserved, consistent with their being the principal determinants of ligand binding specificity.

The $\beta A$-$\alpha 1$ loop, which includes the metal-coordinating $DxSxS$ consensus sequence, has a 2-residue deletion in the $\alpha 2$-I domain, at the beginning of the $\alpha 1$ helix, but is otherwise conserved. The $\alpha 3$-$\alpha 4$ loop has a 1-residue insertion in the $\alpha 2$-I domain, which creates space for the glutamate (in DESNS), as already noted. The $\beta D$-$\alpha 5$ loop is similar in $\alpha 2$-I, $\alpha L$-I, and vWF-A3, but different in $\alpha M$-I, while $\alpha L$-I lacks most of helix $\alpha 5$. The $\beta E$-$\alpha 6$ loop is the site of the principal insertion in the $\alpha 2$-I domain that creates the protruding C-helix. Its conformation is similar in $\alpha L$-I and $\alpha M$-I, but very different in $\alpha 2$-I and vWF-A3. It undergoes substantial rearrangement in the two structures of $\alpha M$-I, creating an acidic pocket in the active conformer, and we suggest that this loop is a major determinant of ligand specificity.

**Collagen Binding**—Integrin $\alpha 2$-I binds several types of fibrillar collagens (types I-VI and XI), and recombinant $\alpha 2$-I domain exhibits specific binding to some, but not all of these
alpha-2 Domain Structure

**Fig. 2.** Sequence alignments of the human integrin 1 domains (α2-I, α3-I, and α5-I) and the vWF-A3, with α-helices and β-sheets indicated for the α2-I domain. Sequences are aligned based on structural superpositions (38). Lowercase letters denote a lack of structural similarity. Sequence identities with a 33.9% sequence identity. The α2-I domain are 26.7% (αm), 24.0% (αL), and 20.3% (vWF-A3). The αm and αL-I domains form a subfamily with 33.9% sequence identity. The α1-I domain sequence alignment is also shown; its structure has not been determined but is likely to be very similar to the α2-I domain.

Collagen IV (34).

A tyrosine residue (Tyr285) projecting prominently into the groove places strong constraints on the position of the collagen helix; in particular, the projecting C-helix, the top of helix α6, and their connecting loop, severely restrict rotations of the collagen about an axis parallel to the glutamate-metal bond. The amino acid side chains in natural collagen sequences would restrict rotations still further. Replacement of the glutamate by the shorter aspartate creates steric clashes in all possible orientations of the collagen. The model predicts that the following I domain residues make contact with the collagen: from the βA-α1 turn (Asn154), from the α3-α4 turn (Asp219 and Leu221), from the βD-α5 turn (Glu256 and His258), and from the C-helix, α6 and C-α6 turn (Tyr285, Asp291, Leu293, and Lys298). The “footprint” on the collagen is about 10 residues long. Mutagenesis data (32) show that individual alanine mutants of two of these residues, Glu256 and Asp291, do not detectably affect collagen binding, but given the large number of potential contacts this may not be surprising. The epitopes for two blocking antibodies have been mapped to Asp256 and Tyr285 (8, 35, 36). These residues lie on the surface of the domain to the side of the MIDAS face, 12–15 Å from the MIDAS motif, consistent with their forming parts of epitopes for blocking antibodies.

Takada’s group (32) have reported conflicting data on collagen binding, showing that in their system binding is cation-independent. However, they also show that binding was completely abrogated by alanine mutagenesis of the MIDAS residues Thr221 and Thr222. A possible resolution of these conflicting data (32) is that individual alanine mutants of two of these residues, Glu256 and Asp291, do not detectably affect collagen binding, but given the large number of potential contacts this may not be surprising. The epitopes for two blocking antibodies have been mapped to Asp256 and Tyr285 (8, 35, 36).

**Fig. 3.** Closeup view of the α2-I domain MIDAS motif. Loops are shown schematically in gray, together with the side chains of the MIDAS motif, the Mg2+ ion (M), and three coordinating water molecules (ω). The side chain of Tyr285, which protrudes from the C-helix, is also shown.

The new C-helix on top of the MIDAS face creates a groove about 25 Å long and 20 Å wide centered on the metal ion, with a tyrosine residue (Tyr285) projecting prominently into the groove. Into this groove we manually docked a collagen triple helix, which was derived from the crystal structure of a collagen-like peptide (30) and modified to contain a glutamate residue to coordinate the MIDAS metal ion (Fig. 5). The shape of the groove places strong constraints on the position of the collagen helix; in particular, the projecting C-helix, the top of helix α6, and their connecting loop, severely restrict rotations of the collagen about an axis parallel to the glutamate-metal bond. The amino acid side chains in natural collagen sequences would restrict rotations still further. Replacement of the glutamate by the shorter aspartate creates steric clashes in all possible orientations of the collagen. The model predicts that the following I domain residues make contact with the collagen: from the βA-α1 turn (Asn154), from the α3-α4 turn (Asp219 and Leu221), from the βD-α5 turn (Glu256 and His258), and from the C-helix, α6 and C-α6 turn (Tyr285, Asp291, Leu293, and Lys298). The “footprint” on the collagen is about 10 residues long. Mutagenesis data (32) show that individual alanine mutants of two of these residues, Glu256 and Asp291, do not detectably affect collagen binding, but given the large number of potential contacts this may not be surprising. The epitopes for two blocking antibodies have been mapped to Asp256 and Tyr285 (8, 35, 36). These residues lie on the surface of the domain to the side of the MIDAS face, 12–15 Å from the MIDAS motif, consistent with their forming parts of epitopes for blocking antibodies.

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while Leu286 is replaced by a tyrosine). The βA-α1 and βD-α5 loops are strictly conserved around the MIDAS motif, while the α3-α4 loop is divergent.

The vWF-A1 and A3 domains have been shown to bind collagens type I and III. Binding to A3 is independent of cation, and the crystal structure of vWF-A3 does not contain a bound metal (24). In addition, mutation of the MIDAS residues do not affect collagen binding. The βE-α6 loop, which contains the C-helix insert in α2-I, is truncated in vWF-A3, so that it is even shorter than in αL-I and αM-I. The result is that the vestigial MIDAS face is quite featureless, suggesting that α2-I and vWF-A3 bind collagen in different ways.

Echovirus Binding—The α2β1 integrin is the receptor for the human pathogen echovirus-1 (4), and the α2-I domain binds directly to the virus (25). Virus binding is cation-independent (35), is not affected by mutations of the MIDAS motif (37), and does not require activation of the integrin (35). King et al. (37) have recently shown that residues 199–201 and 212–216 are involved in virus binding. These residues map to the loops flanking both ends of helix α3, forming part of a flat surface (−20 Å × 30 Å) at one end of the β-sheet, adjacent to but not overlapping the MIDAS face. This location is consistent with biochemical data suggesting that the collagen and echovirus-binding sites are distinct (35). Several monoclonal antibodies block both virus and collagen binding, supporting the idea that the binding sites are in close proximity (35). Part of the epitope for one such antibody, 5E8, has been mapped; it includes residue Tyr216 (36), on the loop between helix α3 and the MIDAS motif.

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