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# Ligand recognition by the I domain-containing integrins

**S. K. Dickeson and S. A. Santoro***

Department of Pathology, Box 8118, Washington University School of Medicine, 660 South Euclid Avenue, St. Louis (Missouri 63110, USA), Fax +1 314 362 3016, e-mail: santoro@labmed.wustl.edu

**Abstract.** Seven of the integrin $\alpha$ subunits described to date, $\alpha_1$, $\alpha_2$, $\alpha_L$, $\alpha_X$, $\alpha_d$, $\alpha_M$ and $\alpha_E$, contain a highly conserved I (or A) domain of approximately 200 amino acid residues inserted near the amino-terminus of the subunit. As the result of a variety of independent experimental approaches, a large body of data has recently accumulated that indicates that the I domains are independent, autonomously folding domains capable of directly binding ligands that play a necessary and important role in ligand binding by the intact integrins. Recent crystallographic studies have elucidated the structures of recombinant $\alpha_M$ and $\alpha_L$ I domains and also delineated a novel divalent cation-binding motif within the I domains (metal ion-dependent adhesion site, MIDAS) that appears to mediate the divalent cation binding of the I domains and the I domain-containing integrins to their ligands.

**Key words.** Adhesion; integrin; I domain; divalent cation; ligand recognition.

## Introduction

Of the 15 integrin $\alpha$ subunits that have been identified to date, the $\alpha_1$, $\alpha_2$, $\alpha_L$, $\alpha_X$, $\alpha_d$, $\alpha_M$ and $\alpha_E$ subunits contain near their amino-termini an autonomously folded domain of approximately 200 amino acids referred to as the I (inserted) domain. A homologous domain, the A domain, is present in von Willebrand factor, cartilage matrix protein, type IV, VII, XII and XIV collagens, and complement proteins factor B, C2, CR3 and CR4. Von Willebrand factor contains three repeats of the A domain. The first and third repeats have been implicated in collagen binding. This observation led to speculation that the ligand-binding activity of the $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins, both of which bind collagens, might also be mediated by their $\alpha$ subunit I domains. Since that time, a substantial body of evidence has accumulated to support the notion that the binding of most, if not all, ligands to I domain-containing integrins is mediated by the integrin $\alpha$ subunit I domain. I domain-mediated ligand binding has been established for five of the seven I domain-containing a subunits. Table 1 summarizes the I domain-containing integrin $\alpha$ subunits, the $\beta$ subunits with which they associate, their ligands and indicates whether the I domain has been implicated in the ligand-binding activity of the integrin.

* Corresponding author.

## The I domain-containing integrins

### $\alpha_1\beta_1$ Integrin

The initial identification and characterization of the $\alpha_1\beta_1$ integrin as a collagen and laminin receptor was an outgrowth of studies with PC12 cells. Pheochromocytoma-derived PC12 cells adhere to laminin and collagen types I, II, III and IV in a $Mg^{2+}$-dependent manner [1]. The ability of a monoclonal antibody, 3A3, to block the adhesion of PC12 cells to collagens and laminin and to promote the retraction of neurites extended on these substrates led to the identification of the $\alpha_1\beta_1$ integrin as a collagen/laminin receptor [2]. Evidence for the involvement of the $\alpha_1$ integrin subunit I domain in

Table 1. The I domain-containing integrins.

| $\alpha$ Subunit | Parent integrin | Substrate | I domain involvement in ligand binding | References |
|---|---|---|---|---|
| $\alpha_1$ | $\alpha_1\beta_1$ | collagen | yes | [3, 4] |
| | | laminin | yes | [3, 4] |
| $\alpha_2$ | $\alpha_2\beta_1$ | collagen | | [20–22, 4] |
| | | laminin | yes | [22, 4] |
| | | collagen C – propeptide | yes | [23] |
| | | echovirus-1 | yes | [19,16] |
| $\alpha_L$ | $\alpha_L\beta_2$ | ICAM-1 | yes | [38] |
| | | ICAM-2 | ? | [34] |
| | | ICAM-3 | yes | [38] |
| $\alpha_X$ | $\alpha_X\beta_2$ | fibrinogen | ? | [55] |
| | | iC3b | yes | [54, 56] |
| $\alpha_d$ | $\alpha_d\beta_2$ | ICAM-3 | ? | [57] |
| $\alpha_M$ | $\alpha_M\beta_2$ | ICAM-1 | yes | [51] |
| | | fibrinogen | yes | [51 |
| | | iC3b | yes | [51] |
| | | Factor X | ? | [46] |
| | | NIF | yes | [50, 52] |
| | | heparin | yes | [49] |
| $\alpha_E$ | $\alpha_E\beta_4$ | ? | ? | [59, 60] |
| | $\alpha_E\beta_7$ | ? | ? | [62–64] |

ligand binding was obtained using another $\alpha_1\beta_1$ integrin function-blocking antibody, 1B3.1. This antibody failed to bind to a functional chimeric $\alpha_1\beta_1$ integrin that consisted of the chicken I domain inserted into the human $\alpha_1$ subunit. Furthermore, mutation of D253 within the chicken I domain to alanine restored 1B3.1 binding and caused a significant decrease in cell adhesion to collagen IV and laminin [3]. Finally, recombinant $\alpha_1$ integrin I domain was shown to bind to types I and IV collagen and laminin in a divalent cation-dependent manner that was inhibited by an $\alpha_1\beta_1$ integrin function-blocking antibody [4]. Thus the adhesion of cells to collagens and laminin via the $\alpha_1\beta_1$ integrin appears to be dependent upon interactions involving the $\alpha_1$ I domain.

## $\alpha_2\beta_1$ Integrin

Recognition that the $\alpha_2\beta_1$ integrin serves as a collagen/laminin receptor grew out of early studies with platelets [5]. Platelets adhere to collagen in a divalent cation-dependent manner [6]. $Mg^{2+}$, $Mn^{2+}$ and several other divalent cations support the adhesion of platelets to collagen. $Ca^{2+}$, which fails to support platelet adhesion to collagen, inhibits the $Mg^{2+}$-dependent adhesion. Following surface iodination and membrane protein solubilization, a 160,000-Da protein was identified as the mediator of cation-dependent adhesion of platelets to collagen [6]. A heterodimeric complex containing the 160,000-Da protein and an additional 130,000-Da protein was purified from platelet membranes by sequential chromatographic steps on concanavalin A-Sepharose and collagen-Sepharose. This complex was shown by immunological means to be identical to VLA-2, or what is now known as the $\alpha_2\beta_1$ integrin [7]. Further evidence that the $\alpha_2\beta_1$ integrin was a $Mg^{2+}$-dependent collagen-binding protein was obtained when the purified integrin was incorporated into liposomes. Like cells expressing the $\alpha_2\beta_1$ integrin, the liposomes bound collagen in an $Mg^{2+}$-dependent manner that was inhibited by $Ca^{2+}$ [7, 8]. Other independent investigations carried out originally with platelets and HT-1080 cells identified the $\alpha_2\beta_1$ integrin as the antigen recognized by an antibody that inhibited cell adhesion to collagen [9–11]. Platelet deficiency of the $\alpha_2\beta_1$ integrin results in platelet unresponsiveness to collagen and a bleeding disorder [12].

Although the $\alpha_2\beta_1$ integrin is widely expressed on several different cell types, its substrate specificity varies. While it is a receptor for both collagen and laminin on endothelial and epithelial cells [13, 14], it binds only collagen when expressed on platelets [8]. The identification of a monoclonal antibody directed against the $\beta_1$ integrin that is capable of converting the $\alpha_2\beta_1$ integrin from a collagen-binding form to a collagen/laminin-binding form provides compelling evidence that the difference between these two forms of the integrin is their conformational state [15]. In addition to the extracellular matrix components collagen and laminin, the $\alpha_2\beta_1$ integrin has been shown to be a receptor for a common human viral pathogen, echovirus-1 [16], and the carboxyl-terminal propeptide of type I collagen [17].

Considerable evidence exists to implicate the I domain of the $\alpha_2$ integrin subunit in the binding of the $\alpha_2\beta_1$ integrin with its ligands. An antiserum directed against an $\alpha_2$ I domain fusion protein expressed in bacteria disrupts the adhesion of endothelial cells to type I collagen and laminin [18]. Using a series of human/bovine $\alpha_2$-integrin subunit chimeras, the epitopes of all known $\alpha_2\beta_1$ function-blocking monoclonal antibodies have been mapped to the I domain [19]. Point mutations at several critical residues within this region of the $\alpha_2$ subunit abolish the adhesion of cells and the binding of recombinant I domain to collagen [19, 20]. Recombinant $\alpha_2$-integrin I domain specifically binds collagen, laminin and the carboxyl-terminal propeptide of type I procollagen in a divalent cation-dependent manner [4, 21–23]. Thus, as is the case for the $\alpha_1\beta_1$ integrin, the I domain of the $\alpha_2$ integrin subunit is the binding site of all known ligands of the $\alpha_2\beta_1$ integrin.

### $\beta_2$ Integrin subfamily

The $\beta_2$ integrins consist of four adhesion receptors whose expression is restricted to white blood cells. Unlike the $\beta_1$ integrins, all known $\beta_2$ integrins contain an I domain within their $\alpha$ subunits. Within this group are the $\alpha_L\beta_2$, $\alpha_M\beta_2$, $\alpha_X\beta_2$ and $\alpha_d\beta_2$ integrins. An inherited $\beta_2$-integrin deficiency has provided evidence for the importance of the $\beta_2$ integrins for normal immune function. Leukocyte adhesion deficiency (LAD) is a disease in which the expression of the $\beta_2$-integrin subunit is either deficient or absent [24]. The loss (or decreased expression) of the $\beta_2$ integrins in LAD patients results in recurring bacterial infections, impaired neutrophil mobility and respiratory burst, abnormal neutrophil phagocytosis and other related defects. Transfection of the $\beta_2$-integrin subunit complimentary DNA (cDNA) into B lymphoblastoid cells from LAD patients restored normal levels of the $\alpha_L\beta_2$ integrin to the cell surface [25]. Homotypic adhesion and adhesion to intercellular adhesion molecule-1 (ICAM-1) was also restored in the $\beta_2$ transfected cells. These experiments verified that the LAD defect is in the $\beta_2$-integrin subunit.

### $\alpha_L\beta_2$ Integrin

The $\alpha_L\beta_2$ integrin, also referred to as LFA-1 (lymphocyte function-associated antigen 1), is expressed on all leukocytes with the exception of some macrophages [26]. Although identified by screening for monoclonal antibodies that inhibited CTL (cytolytic T lymphocyte)-mediated killing [27], the $\alpha_L\beta_2$ integrin is also required for many other leukocyte functions, including T-helper and B-lymphocyte responses, natural killing, antibody-dependent cytotoxicity mediated by monocytes and granulocytes, and adhesion of leukocytes to endothelial cells, fibroblasts and epithelial cells [28].

The first counterreceptor identified for the $\alpha_L\beta_2$ integrin, ICAM-1, was identified by screening for monoclonal antibodies that inhibited the aggregation of $\alpha_L\beta_2$-positive cells [29]. ICAM-1 expression is rapidly induced by inflammatory cytokines [30], and analysis of its cDNA sequence has revealed that it is a member of the immunoglobulin supergene family with five immunoglobulin-like domains [31, 32]. The identification of ICAM-1 as a ligand for the $\alpha_L\beta_2$ integrin provided the first example of an interaction between a member of the integrin family and a member of the immunoglobulin superfamily.

The adhesion of inflammatory cells to endothelial cell monolayers occurs by both $\alpha_L\beta_2$-dependent and $\alpha_L\beta_2$-independent mechanisms. $\alpha_L\beta_2$-Dependent adhesion is further subdivided into $\alpha_L\beta_2$-dependent/ICAM-1-dependent and $\alpha_L\beta_2$-dependent/ICAM-1-independent mechanisms [33]. This led to the search for additional ligands for the $\alpha_L\beta_2$ integrin. ICAM-2, identified by functional screening of a cDNA library prepared from endothelial cells [34], is also a member of the immunoglobulin superfamily. ICAM-2 contains two immunoglobulin-like domains. These domains are similar (34% identity) to immunoglobulin-like domains 1 and 2 of ICAM-1. Unlike ICAM-1, the basal expression of ICAM-2 on endothelial cells is high, and its expression is not induced further by lipopolysaccharide [34].

The observation that a mixture of blocking monoclonal antibodies to ICAM-1 and ICAM-2 failed to inhibit completely the adhesion of some T- and B-lymphocyte cell lines to purified $\alpha_L\beta_2$ integrin was the impetus for a search for a monoclonal antibody that could inhibit the remaining adhesion, thus identifying a third ligand for the $\alpha_L\beta_2$ integrin [35]. Such an antibody was identified; adhesion of resting lymphocytes to purified $\alpha_L\beta_2$ integrin was almost entirely mediated by ICAM-3. Cloning of the ICAM-3 cDNA revealed that it is also closely related to ICAM-1 [36, 37]. Like ICAM-1, ICAM-3 contains five immunoglobulin-like domains. Unlike ICAM-1, however, ICAM-3 is constitutively expressed on resting leukocytes, leading to the speculation that it may be important for the initiation of the immune response [36].

Evidence for the involvement of the $\alpha_L$ I domain in binding to ICAM-1 and ICAM-3 has been obtained. A screen of 21 $\alpha_L$ and 10 $\beta_2$ monoclonal antibodies identified a single $\alpha_L$ antibody, MEM-83, that was capable of enhancing the binding of T cells to purified ICAM-1. Using a series of $\alpha_L$-deletion mutants, the MEM-83 activation epitope was mapped to the I domain [38]. In addition, purified recombinant $\alpha_L$-integrin I domain binds to ICAM-1 and inhibits $\alpha_L\beta_2$-integrin-dependent T cell adhesion to ICAM-1. The epitopes of 18 out of

20 $\alpha_L\beta_2$ integrin-function blocking antibodies map to the I domain [39]. Finally, a screen of 19 $\alpha_L$ and 10 $\beta_2$ monoclonal antibodies identified two $\alpha_L$ antibodies that inhibited T-cell adhesion to ICAM-3 [40]. The epitopes of both of these antibodies were mapped to the I domain. Interestingly, neither of these antibodies inhibited T-cell adhesion to ICAM-1. Conversely, the activating antibody MEM-83 was incapable of stimulating T-cell adhesion to ICAM-3. The authors speculate that alternative forms of the I domain may be present on the cell surface after activation and that the activating antibody is capable of inducing or stabilizing the ICAM-1-binding form, while the inhibitory antibodies interfere with the ICAM-3-binding form. In any event, these studies provide data that demonstrate the importance of the $\alpha_L$ I domain with respect to ligand binding of the $\alpha_L\beta_2$ integrin.

### $\alpha_M\beta_2$ Integrin

The adhesive function of the $\alpha_M\beta_2$ integrin, or Mac-1, was identified using a monoclonal antibody (M1/70) from a panel of antibodies that reacted with mouse cell surface antigens [41]. The distribution of the $\alpha_M\beta_2$ integrin is more restricted than that of the $\alpha_L\beta_2$ integrin; it is found almost exclusively on myeloid cells [42]. The ability of antibody M1/70 to inhibit rosetting of iC3b-coated erythrocytes to murine macrophages and human polymorphonuclear leukocytes identified the $\alpha_M\beta_2$ integrin as the type three complement receptor [43]. Activated neutrophils adhere to stimulated endothelial cells in a manner dependent on the $\alpha_M\beta_2$ integrin on the neutrophil and ICAM-1 on the endothelial cells, demonstrating that ICAM-1, a ligand for the $\alpha_L\beta_2$ integrin, also binds the $\alpha_M\beta_2$ integrin [44]. A role of the $\alpha_M\beta_2$ integrin/ICAM-1 interaction is to facilitate firm attachment of leukocytes to the endothelium following rolling along the endothelium at sites of inflammation [45]. Stationary adhesion is a prerequisite for neutrophil extravasation at sites of inflammation. Additional ligands for the $\alpha_M\beta_2$ integrin include factor X [46], fibrinogen [47, 48] and heparin [49]. The hookworm-derived glycoprotein NIF (neutrophil inhibitory factor) exerts its effects of blocking both the adhesion of activated neutrophils to vascular endothelial cells and the release of $H_2O_2$ from activated neutrophils by virtue of a high-affinity interaction with the $\alpha_M\beta_2$ integrin [50].

Most of the $\alpha_M\beta_2$ integrin/ligand interactions that have been characterized to date are clearly due to recognition of the ligands by the $\alpha_M$-integrin subunit I domain. The epitopes recognized by monoclonal antibodies that block the binding to $\alpha_M\beta_2$ of iC3b, ICAM-1, fibrinogen and a fourth ligand that facilitates neutrophil homotypic adhesion all map to the $\alpha_M$ integrin I domain [51]. NIF binds recombinant $\alpha_M$ I domain with high affinity and in a specific manner. The binding is blocked by a monoclonal antibody directed against the $\alpha_M$ I domain [52].

### $\alpha_X\beta_2$ Integrin

Expression of the $\alpha_X\beta_2$ integrin, previously referred to as p150,95, is mainly restricted to myeloid cells [53]. The isolation of activated U937 (a monocytic cell line) cell surface proteins by affinity chromatography on immobilized iC3b led to the identification of the $\alpha_X\beta_2$ integrin as an iC3b receptor [54]. Tumour necrosis factor-$\alpha$-stimulated polymorphonuclear leukocytes adhere to fibrinogen in a manner blocked by two monoclonal antibodies directed against the $\alpha_X$-integrin subunit [55]. Thus fibrinogen is a ligand for both the $\alpha_M\beta_2$ and the $\alpha_X\beta_2$ integrins. The epitope recognized by an $\alpha_X$-integrin monoclonal antibody capable of blocking the adhesion of cells and the binding of purified $\alpha_X\beta_2$ integrin to iC3b has been mapped to the $\alpha_X$-integrin subunit I domain, showing the importance of this I domain in the ligand recognition and binding by the $\alpha_X\beta_2$ integrin [56].

### $\alpha_d\beta_2$ Integrin

The most recently identified member of the $\beta_2$-integrin subfamily is the $\alpha_d\beta_2$ integrin [57]. The $\alpha_d$ subunit is more similar to the $\alpha_M$ and $\alpha_X$ integrin subunits than to the $\alpha_L$ subunit. The distribution of the $\alpha_d\beta_2$ integrin is somewhat different than that of the $\alpha_M\beta_2$ and $\alpha_X\beta_2$ integrins. It is expressed on macrophages and granulocytes in the splenic red pulp, on lipid-laden macrophages in aortic fatty streaks and to a lesser extent on peripheral blood leukocytes. Flow cytometry and solid-phase adhesion assays were used to compare the binding of the $\alpha_d\beta_2$ integrin to ICAM-1 and ICAM-3. Both methods demonstrated that the $\alpha_d\beta_2$ integrin preferentially bound ICAM-3. Involvement of the $\alpha_d$-integrin subunit I domain in ligand recognition and binding has not been demonstrated.

### $\alpha_E\beta_4$ and $\alpha_E\beta_7$ integrins

The screening of monoclonal antibodies prepared against the human pancreatic cell line FG led to the discovery of the $\alpha_E\beta_4$-integrin [58, 59]. This integrin is localized to the basal surface of keratinocytes, and anti-$\beta_4$-integrin antibodies block the adhesion of keratinocytes to purified laminin [60]. Thus the $\alpha_E\beta_4$ integrin appears to mediate the adhesion of keratinocytes to basal lamina. Specific ligands for the $\alpha_E\beta_4$ integrin have not been identified.

The $\alpha_E\beta_7$ integrin was also identified by reactivity with a monoclonal antibody [61]. This integrin, along with $\alpha_4\beta_7$,

is expressed on intestinal intraepithelial lymphocytes. The $\alpha_E\beta_7$ integrin, in conjunction with the $\alpha_L\beta_2$ integrin, has been shown to mediate the adhesion of T lymphocytes to epithelial cells [62]. The epithelial cell counterreceptor for the $\alpha_E\beta_7$ integrin has been identified as E-cadherin [63]. Cloning of the $\alpha_E$-integrin subunit revealed the presence of an I domain, and an extra 55 amino acid domain just amino-terminal to the I domain [64]. This extra domain is highly negatively charged and contains a protease cleavage site. Among the $\alpha$-integrin subunits, this domain is unique to $\alpha_E$. Northern blot analysis revealed that the $\alpha_E$-integrin messenger RNA (mRNA) is restricted to mucosal lymphocytes. As yet there is no evidence for the involvement of the $\alpha_E$ I domain in ligand binding.

## I domain structure

### Protein structure

The primary sequence of each of the integrin $\alpha$-subunit I domains is shown in figure 1. Even a cursory examination of the sequences reveals that the integrin I domains constitute a family of closely related proteins with a high degree of sequence identity and/or similarity. Recent efforts by two groups have led to solution of crystal structures for the $\alpha_M$- and $\alpha_L$-integrin subunit I domains [65–68]. These studies have resulted in dramatic new insights into I domain structure and function.

As the major structural features identified were common to both the $\alpha_M$ and $\alpha_L$ I domains, only the $\alpha_M$ structure is described in detail. A stereoscopic ribbon diagram of the $\alpha_M$ domain is presented in figure 2. The I domain is composed of alternating amphipathic $\alpha$ helices and hydrophobic $\beta$ strands. The five parallel and one antiparallel $\beta$ strands form a central sheet that is surrounded by the seven $\alpha$ helices. The I domain structure is an example of the 'Rossmann' dinucleotide, or doubly-wound fold. A crevice is formed along the top of the $\beta$ sheet. In all other proteins exhibiting this type of folded structure, the apical crevice functions as a ligand-binding site. Additional observations described below suggest that this is probably also true for the I domains.

### Divalent cation binding

In accordance with observations from an earlier experimental study suggesting that the I domain contained a novel divalent cation-binding motif [69], a single divalent cation binding site was located in the $\alpha_M$ and $\alpha_L$ I domain crystal structures in the crevice at the top of the $\beta$ sheet. The structural basis of $Mg^{2+}$ binding to the $\alpha_M$ I domain will be used as an example to facilitate an initial detailed discussion of the metal-binding properties of the I domain (fig. 3a).

$Mg^{2+}$ has sites for six coordinating ligands arranged in an octahedral geometry. Three of the six $Mg^{2+}$ coordination sites in the I domain are provided by the hydroxyl oxygen atoms of S142, S144 and T209 of $\alpha_M$. Two additional coordination ligands are provided by water molecules w1 and w2. w2 is also hydrogen bonded to a carbonyl oxygen of E244. The two aspartate residues, D140 and D242, previously implicated in metal binding by mutagenesis studies, appear to participate indirectly by hydrogen bonding to a water molecule (w1) and hydroxyl side chains of S142 and S144, respectively. Somewhat surprisingly, the sixth and apical coordination ligand was provided by E314 of a neighbouring I domain molecule in the crystal. It seems likely that, in solution, this coordinating ligand is provided by water. Given the position of the metal ion in the putative ligand-binding crevice, it is attractive to consider that an acidic side chain from the ligand might provide a site in the ligand-bound form of the I domain.

The $\alpha_M$ I domain has also been crystallized in the presence of $Mn^{2+}$ [66]. The profound structural differences observed between the $Mn^{2+}$- and $Mg^{2+}$-occupied forms of the I domain may shed light on the structural basis underlying some divalent cation-dependent alterations in I domain ligand-binding specificity. For example, $Mg^{2+}$ effectively supports binding of the $\alpha_2$-integrin I domain to collagen and only poorly supports binding to laminin, whereas $Mn^{2+}$ effectively supports binding to both ligands [22, 70]. The manner in which $Mn^{2+}$ is coordinated in the $\alpha_M$ I domain is shown in figure 3b. The position of the divalent cation is shifted by 2.3 Å, relative to its position in the $Mg^{2+}$-loaded form of the I domain. The two serine residues continue to provide coordinating ligands, as in the $Mg^{2+}$ form. However, in the $Mn^{2+}$ form, the coordinating side chain of T209 is replaced by the D242 side chain. The three remaining sites are provided by water molecules. The changes in coordination pattern have marked effects on the protein structure. The C-terminal $\alpha 7$ helix (see fig. 2) moves 10 Å up the side of the I domain molecule, with burial of F302, which was completely exposed in the $Mg^{2+}$ structure. These changes necessitate additional movements of three connecting segments, subsequent burial of F275, which was also exposed in the $Mg^{2+}$ structure, and consequent repositioning of D242 so that it is now capable of supplying a coordination ligand to the $Mn^{2+}$. Although the $\alpha_M$ I domain structure is profoundly dependent upon the identity of the bound divalent cation, this does not appear to be the case for the $\alpha_L$ I domain. Structures of the $\alpha_L$ I domain have recently been solved for crystals containing either $Mg^{2+}$ or $Mn^{2+}$, as well as for I domains free of metal [68]. Although the general

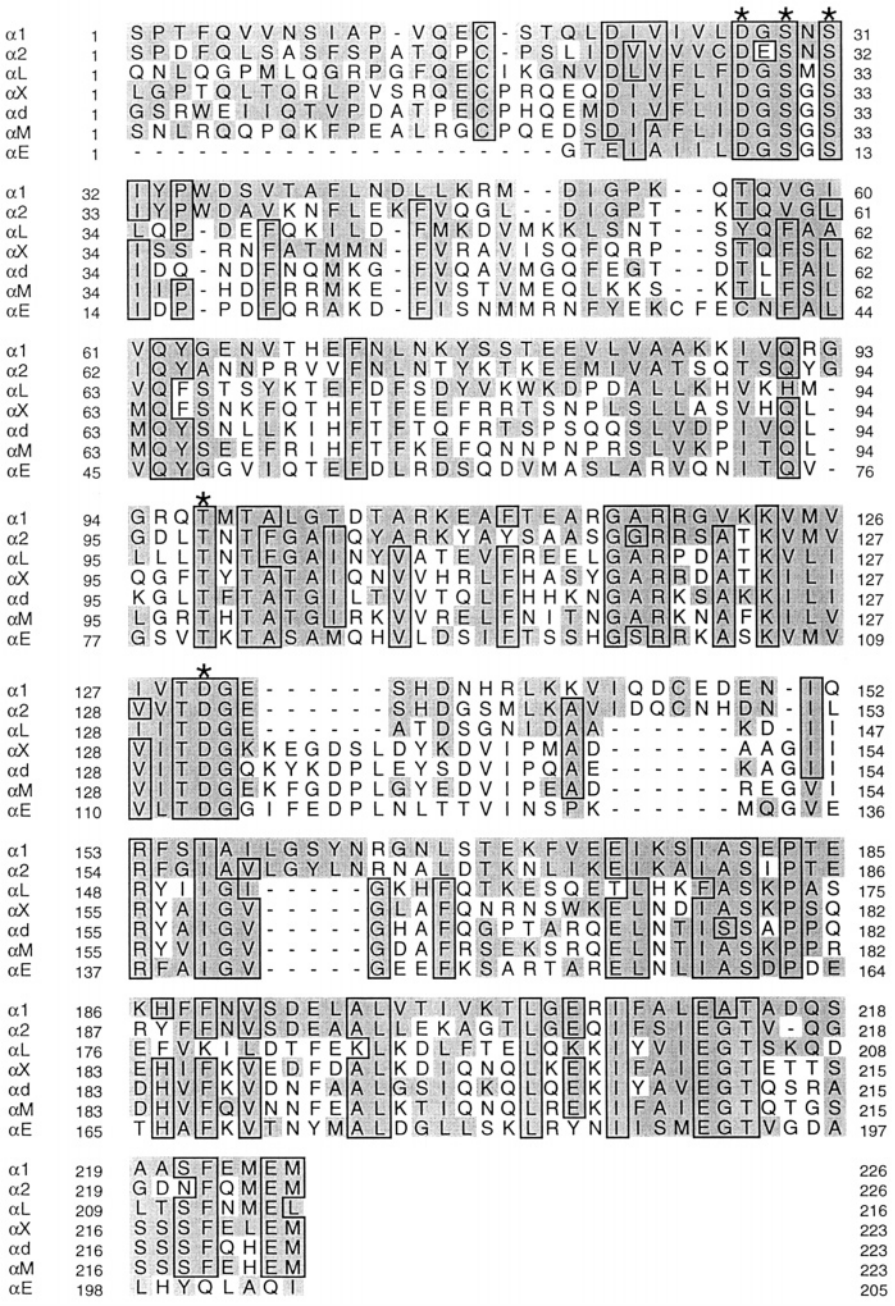

Figure 1. Comparison of amino acid sequences of the I domains from the seven I domain-containing integrins. Regions of identity are boxed; regions of identity or conserved similarity are shaded. Positions of the conserved residues constituting the MIDAS motif are indicated by asterisks.

features of I domain structure from the study of the $\alpha_M$ domain clearly apply to the $\alpha_L$ I domain, the $\alpha_L$ structure was not dependent upon the presence or identity of divalent cation. The $\alpha_L$ data suggest that the divalent cation dependence of ligand binding and specificity arises either because of direct interactions of ligands with the metal, or because the metal is required to promote a favourable quaternary arrangement of the integrin [68].

A recent study of divalent cation binding to the recombinant $\alpha_2$-integrin subunit I domain exploited $Tb^{3+}$, a fluorescent probe of protein divalent cation-binding sites, to examine the relative affinities of divalent cations for the I domain (S. K. Dickeson et al., unpub-

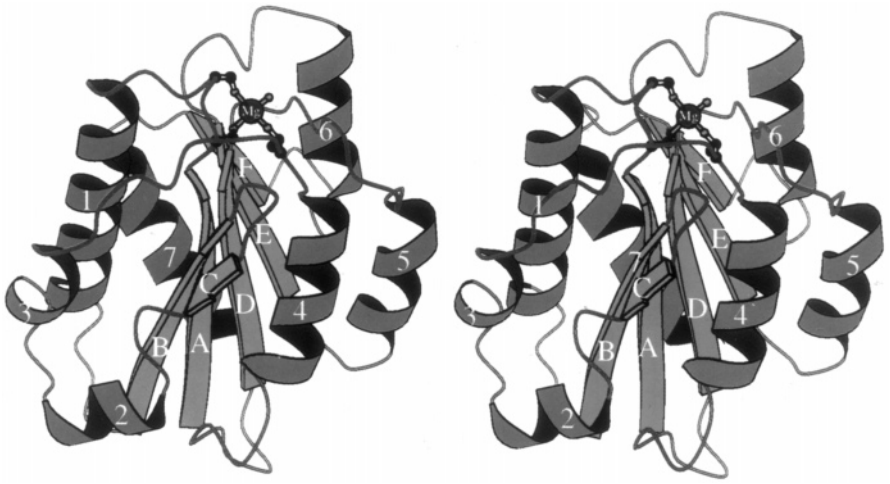


Figure 2. Schematic stereoribbon diagram of the $\alpha_M$ integrin subunit I domain structure. Reprinted with permission from: Lee J.-O., Rieu P., Arnaout M. A. and Liddington R. (1995). Crystal structure of the A domain from the $\alpha$ subunit of integrin CR3 (CD11b/CD18). Cell **80:** 631–638, © Cell Press, 1997.

lished observations). As expected from earlier studies, $Ca^{2+}$ did not compete with $Tb^{3+}$ for binding. Although $Mg^{2+}$ and $Mn^{2+}$ both competed with $Tb^{3+}$ for binding, $Mn^{2+}$ was a much more effective competitor than $Mg^{2+}$, suggesting that the $\alpha_2$-integrin I domain divalent cation-binding site prefers $Mn^{2+}$ over $Mg^{2+}$.

The above studies have placed the divalent cation-binding properties of the integrin I domains on a very solid structural foundation. Metal ion binding is mediated by the conserved DXSXS sequence, residues 140–144 in $\alpha_M$ and homologous regions in other I domains, T209 and D242 of $\alpha_M$ and their equivalents in other I domains. Unlike the EF-hand metal-binding motifs present elsewhere in integrin a subunits, the I domain metal-binding motif is constructed from non-contiguous residues. The DXSXS motif and the equivalents of T209 and D242 of $\alpha_M$ are conserved in all integrin I domains. The locations of these conserved metal-binding residues are indicated by asterisks in figure 1. The function of these residues in divalent cation and ligand binding is supported by rather extensive mutagenesis studies of the $\alpha_M$-, $\alpha_L$- and $\alpha_2$-integrin subunits [19, 20, 69, 71]. Although mutagenesis of T221 of the $\alpha_2$ I domain was initially thought to impair direct interaction with collagen [20], T221 of $\alpha_2$ is homologous to T209 of $\alpha_M$. The divalent cation dependence of ligand binding to integrins and their I domains, as well as the location of the motif in a putative ligand-binding crevice of the I domain, has led to its designation as a MIDAS (metal ion-dependent adhesion site) motif [65].

**Ligand binding**

Rapid and dramatic progress has been made in identifying potent, low molecular weight antagonists of ligand binding for many of the integrins that lack I domains. Similar attempts to identify small inhibitory peptides derived from the ligands of I domain-containing integrins have met with only limited success, resulting in the identification of only weakly inhibitory sequences [72–74]. It now seems likely that most I domain-containing integrins will be found to recognize structurally and conformationally complex determinants within their ligands that are not effectively mimicked by short linear synthetic peptides.

Although abundant evidence has now accumulated to support the roles of the I domain and its MIDAS motif in ligand binding to I domain-containing integrins, a substantial body of data indicates that amino acid residues and structural regions other than those mediating divalent cation binding are essential for ligand binding and that the different ligands for a given I domain may interact with the I domain somewhat differently. Only a few pertinent examples are cited to support these concepts.

The human $\alpha_2$ integrin and I domain bind collagens, laminin and echovirus 1 [75]. Antibodies that inhibit binding to collagen and laminin have no effect on echovirus binding and vice versa, despite the fact that all of the antibodies map to epitopes within the I domain [19, 76]. Furthermore, although the human and mouse $\alpha_2$ integrin subunits are 84% identical, the human integrin binds echovirus, whereas the mouse integrin does not. Insertion of the human $\alpha_2$-integrin I domain into the murine $\alpha_2$-integrin subunit to create a murine/human

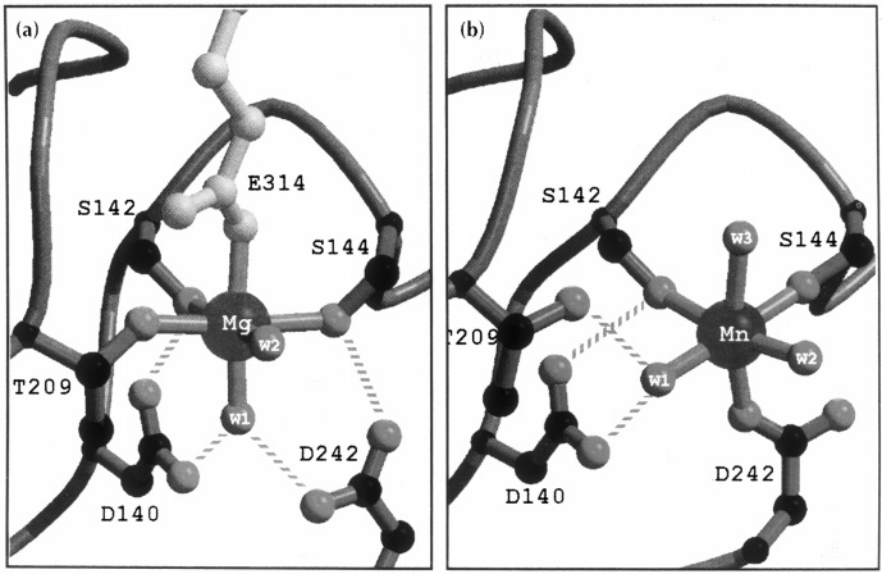


Figure 3. High-resolution comparison of divalent cation coordination and I domain structure of the $Mg^{2+}$ (*a*) and $Mn^{2+}$ (*b*) forms of the $\alpha_M$-integrin subunit I domain. Water molecules are labelled w1–w3. Selected hydrogen ions are shown by dashed lines. The identity of coordinating amino acid side chains are indicated by a single letter code. Reprinted with permission from: Lee J. -O., Bankston L. A., Arnaout M. A. and Liddington R. C. (1995). Two conformations of the integrin A-domain (I-domain): a pathway for activation? Structure **3:** 1333–1340, © Current Biology Ltd., 1997.

chimeric $\alpha_2$ subunit containing the human I domain conferred virus-binding activity upon the chimera [76]. Although the $\alpha_2$-integrin I domain binds both collagens and laminin, type I collagen binding was markedly enhanced by the addition of the contiguous first EF-hand motif to the recombinant I domain, whereas laminin binding was unaffected by this addition [22, 70].

As indicated earlier, the I domain of the $\alpha_M$ subunit is a major recognition site for four distinct $\alpha_M\beta_2$ ligands: iC3b, fibrinogen, ICAM-1 and an uncharacterized counterreceptor responsible for neutrophil homotypic adhesion. The use of a battery of monoclonal antibodies that recognized distinct epitopes within the I domain and blocked the binding of one or more ligands to the integrin led to the conclusion that ligand recognition sites in the I domain were overlapping but nonidentical, as individual $\alpha_M\beta_2$-ligand pairs were distinguishable by discrete patterns of antibody inhibition [51]. A recent mutagenesis experiment reinforces this conclusion. A switch of the R281QELNTI sequence in helix 6 of the $\alpha_M$ I domain to the corresponding QETLHKF sequence of the $\alpha_L$ subunit completely inhibited adhesion to fibrinogen, but had no effect on iC3b or NIF binding by the integrin [77]. A very recent study has localized the NIF-binding site to a limited region on the surface of the $\alpha_M$ I domain composed of P147-R152, P201-K217 and D248-R261 [78]. These regions are located in the $\alpha$1 helix, the connecting segment between the $\alpha$3 and $\alpha$4 helices, and helix $\alpha$5, respectively, in the model shown in figure 2. When these regions of the $\alpha_M$ I domain were introduced into the $\alpha_X$ I domain, the $\alpha_X$ I domain acquired high-affinity NIF-binding activity.

Similar but less extensive studies have been carried out to examine the binding of $\alpha_L\beta_2$ to ICAM-1 and ICAM-3. Two monoclonal antibodies that selectively blocked binding to ICAM-3 but had no effect on binding to ICAM-1 were identified, both of which recognized epitopes within the I domain [40]. A third antibody recognizing a distinct I-domain epitope activated $\alpha_L\beta_2$ to enhance binding to ICAM-1 but not ICAM-3.

## Summary and conclusions

A large body of data obtained during the past few years using multiple independent experimental approaches has unambiguously established a crucial role for the I domain in the binding of ligands by the intact I domain-containing integrins. A novel type of divalent cation binding site, the MIDAS motif, has been defined within the I domain and is thought to play a critical role in the divalent cation-dependent binding of I domains and I domain-containing integrins to their ligands. Our recent observations suggest that binding of at least some ligands to the $\alpha_2$-integrin I domain results in displacement of the divalent cation from the integrin (S. K. Dickeson et al., unpublished observations).

Recent crystallographic studies of the $\alpha_M$ and $\alpha_L$ I domains have considerably elevated our understanding of I domain structure and have provided clues to design more sophisticated approaches to define the detailed molecular interactions involved in ligand binding to the I domains. The next large step in our understanding of these biologically important receptor-ligand interactions will likely be a three-dimensional view of an I domain-ligand complex.

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