Minireview

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Ligand Binding to Integrins*

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The "integrin" terminology was applied in a 1987 review article (1) to describe a family of structurally, immunochemically, and functionally related cell-surface heterodimeric receptors, which *integrated* the extracellular matrix with the intracellular cytoskeleton to mediate cell migration and adhesion. The three original β subunits (β_1, β_2, and β_3) identified have now expanded to eight, and the number of α subunits stands at 17. These subunits interact noncovalently in a restricted manner to form more than 20 family members. The diversity of integrins is expanded further by alternative splicing, post-translational modifications, and interactions with other cell-surface and intracellular molecules (2–4). The number of integrins and the remarkable breadth of their cellular distribution support the statement that the phenotype of virtually every cell is uniquely influenced by its display of integrins. Over the past 13 years, more than 14,000 scientific articles have dealt with various aspects of integrin biology and almost 1,000 have appeared in the *Journal of Biological Chemistry*. This article examines a central aspect of integrin biology: ligand recognition and the structural basis for this function.

Ligand Repertoire and Recognition

Repertoire Considerations—A hallmark of the integrins is the ability of individual family members to recognize multiple ligands. Indeed, the extent of the integrin family pales in comparison with the number of their ligands. Table I summarizes the major extracellular ligands of integrins; the listing is undoubtedly incomplete. The list includes a large number of extracellular matrix proteins (bone matrix proteins, collagens, fibronectins, fibrinogen, laminins, thrombospondins, vitronectin, and von Willebrand factor), reflecting the primary function of integrins in cell adhesion to extracellular matrices. Many "counter-receptors" are ligands, reflecting the role of integrins in mediating cell-cell interactions. Included are numerous microorganisms, which utilize integrins to gain entry into cells. There are direct and multiple linkages between integrins and host defense systems, created by their recognition of hemostatic and complement factors. The preference of any given integrin among its ligands is determined by relative affinity, availability within a specific microenvironment, and the conformational state of the ligand, which controls exposure of its integrin recognition sequence.

Integrin Recognition Sequences—A primary goal of many structure-function analyses in the integrin field has been the reduction of macromolecular ligands to minimal recognition sequences. This endeavor has been highly successful, and many bioactive amino acid sequences have been teased out of large extracellular matrix proteins (5). The prototypic example is the RGD sequence. RGD was originally identified as the sequence in fibronectin that engages *the fibronectin receptor*, integrin $\alpha_5\beta_1$, but now is known to serve as a recognition motif in multiple ligands for several different integrins (see Table II). Although RGD peptides inhibit ligand binding to integrins with an RGD *recognition specificity* (Table II), these receptors can discriminate among RGD-containing ligands. The context of the RGD sequence (flanking residues, three-dimensional presentation, and individual features of the integrin binding pockets) determine whether productive interactions occur (6). As an illustrative example of the nuances of the RGD recognition specificity, whereas both of the β_3 integrins, $\alpha_{IIb}\beta_3$ and $\alpha_V\beta_3$, recognize fibrinogen, which contains multiple RGD sequences, and RGD peptides inhibit the binding of fibrinogen to these integrins, both integrins can recognize other sequences in fibrinogen (7, 8). Thus, recognition of this seemingly simple tripeptide sequence is complex. A second set of fibronectin sequences also has received considerable attention: those recognized by $\alpha_4\beta_1$. Originally, the CS-1 sequence, which resides in an alternatively spliced segment of fibronectin, was determined to be a recognition site, but now several additional fibronectin sequences have been identified that interact with $\alpha_4\beta_1$ (9–11). Multiple recognition sites also exist in fibrinogen for $\alpha_M\beta_2$ (12). Two generalizations can be derived from these examples: 1) integrin recognition specificities can often be reduced to small peptide sequences; and 2) peptide inhibition studies need to be complemented with other approaches to assess the role of specific sequences in ligand recognition by integrins.

Divalent Cations and Ligand Binding to Integrins

Integrins as Metalloproteins—Each integrin heterodimer contains 3–5 divalent cation binding sites of relatively low affinity (μM^{-1} to mM^{-1}), and the bound cations exert profound effects on integrin function. Collectively, these bound divalent ions can act as effectors, promoting ligand binding; as antagonists, inhibiting ligand binding; and as selectors, changing the ligand binding specificity. One proposal to explain the influential role of cations on integrin function is that ligand and divalent cation share a common binding pocket on the integrin. This hypothesis was supported by data showing that RGD ligands could displace two receptor-bound metal ions and that divalent ion and RGD peptide could bind, in a mutually exclusive manner, a peptide from the β_3 subunit (13). Thus, a "displacement model" was proposed, in which RGD ligands initially form a ternary complex with receptor-bound divalent ion; then, as contacts between RGD and integrin stabilize, the divalent ion may be displaced. Recently, this model was extended to other integrins (14); collagen displaced Tb^{3+} bound to the I domain of the α_2 subunit. Dissection of the ligand binding reaction into ligand association and dissociation steps provided further insights into the roles of divalent ions in integrin function (15). Using surface plasmon resonance, the β_3 integrins were shown to contain two classes of ion binding sites. One class must be occupied for ligand to bind, ligand-competent (LC)[1] sites; and the second class has an inhibitory effect on ligand binding, I sites. The I site(s) display specificity for Ca^{2+} and increase the rate of ligand dissociation. Because the I sites are allosteric to the ligand binding pocket, they can bind Ca^{2+} even when ligand is prebound to integrin, providing a potential mechanism for the release of pre-existing cell-matrix contacts. Thus, it is the coordination between the LC and I cation binding sites that regulates the ligand binding event.

The Structural Basis of Divalent Ion Regulation—There are at least two structurally distinct classes of ion binding motifs within

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[1] The abbreviations used are: LC, ligand competent; MIDAS, metal ion-dependent adhesion site; mAb, monoclonal antibody; ICAM, intracellular adhesion molecule; VCAM, vascular cell adhesion molecule; MAdCAM, mucosal addressin cell adhesion molecule.

TABLE I
Integrin extracellular ligands

Ligand	Integrin
Adenovirus penton base protein	$\alpha_v\beta_3$, $\alpha_v\beta_5$
Bone sialoprotein	$\alpha_v\beta_3$, $\alpha_v\beta_5$
Borrelia burgdorferi	$\alpha_{IIb}\beta_3$
Candida albicans	$\alpha_M\beta_2$
Collagens	$\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_{11}\beta_1$, $\alpha_{Ib}\beta_3$
Denatured collagen	$\alpha_5\beta_1$, $\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$
Cytotactin/tenascin-C	$\alpha_8\beta_1$, $\alpha_9\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_6$
Decorsin	$\alpha_{IIb}\beta_3$
Disintegrins	$\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$
E cadherin	$\alpha_E\beta_7$
Echovirus 1	$\alpha_2\beta_1$
Epiligrin	$\alpha_3\beta_1$
Factor X	$\alpha_M\beta_2$
Fibronectin	$\alpha_2\beta_1$, $\alpha_3\beta_1$, $\alpha_4\beta_1$, $\alpha_4\beta_7$, $\alpha_5\beta_1$, $\alpha_8\beta_1$, $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$, $\alpha_v\beta_8$, $\alpha_{IIb}\beta_3$
Fibrinogen	$\alpha_5\beta_1$, $\alpha_M\beta_2$, $\alpha_v\beta_3$, $\alpha_x\beta_2$, $\alpha_{IIb}\beta_3$
HIV Tat protein	$\alpha_v\beta_3$, $\alpha_v\beta_5$
iC3b	$\alpha_M\beta_2$, $\alpha_x\beta_2$
ICAM-1	$\alpha_L\beta_2$, $\alpha_M\beta_2$
ICAM-2,3,4,5	$\alpha_L\beta_2$
Invasin	$\alpha_3\beta_1$, $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_6\beta_1$
Laminin	$\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_6\beta_1$, $\alpha_7\beta_1$, $\alpha_6\beta_4$, $\alpha_v\beta_3$
MAdCAM-1	$\alpha_4\beta_7$
Matrix metalloproteinase-2	$\alpha_v\beta_3$
Neutrophil inhibitory factor	$\alpha_M\beta_2$
Osteopontin	$\alpha_v\beta_3$
Plasminogen	$\alpha_{IIb}\beta_3$
Prothrombin	$\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$
Sperm fertilin	$\alpha_6\beta_1$
Thrombospondin	$\alpha_3\beta_1$, $\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$
VCAM-1	$\alpha_4\beta_1$, $\alpha_4\beta_7$
Vitronectin	$\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_{IIb}\beta_3$
von Willebrand factor	$\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$

TABLE II
Integrin recognition sequences

Recognition sequence	Ligand	Integrin
RGD	Adenovirus penton base protein, bone sialoprotein, collagen, decorsin, disintegrins, fibrinogen, fibronectin, prothrombin, tenascin, thrombospondin, vitronectin, von Willebrand factor	$\alpha_3\beta_1$, $\alpha_5\beta_1$, $\alpha_8\beta_1$, $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$, $\alpha_{IIb}\beta_3$
HHLGGAKQAGDV	γ-Chain of fibrinogen	$\alpha_{IIb}\beta_3$
GPR	α-Chain of fibrinogen	$\alpha_x\beta_2$
P1 peptide	γ-Chain of fibrinogen	$\alpha_M\beta_2$
P2 peptide	γ-Chain of fibrinogen	$\alpha_M\beta_2$
AEIDGIEL	Tenascin	$\alpha_9\beta_1$
QIDS	VCAM-1	$\alpha_4\beta_1$
LDT	MAdCAM-1	$\alpha_4\beta_7$
CS-1 peptide	Fibronectin	$\alpha_4\beta_1$, $\alpha_4\beta_7$
CS-5 peptide	Fibronectin	$\alpha_4\beta_1$
IDAPS	Fibronectin	$\alpha_4\beta_1$
ICAM peptides	ICAM-1, -2, -3	$\alpha_L\beta_2$, $\alpha_M\beta_2$
DL*XX*L	Tenascin	$\alpha_v\beta_6$
GFOGER[a]	Collagen	$\alpha_1\beta_1$, $\alpha_2\beta_1$

[a] O, hydroxyproline.

integrins. A series of EF-hand-like domains are present in each of the integrin α subunits (16). The integrin EF-hand loops lack a glutamate that is found at the 12th position in virtually all other EF-hand loops and is one of the ligands for Ca^{2+}. The absence of this residue in integrins is likely to explain their lower affinity and selectivity for divalent ions. Two studies have examined the ion and ligand binding function of recombinant fragments containing the integrin EF-hands. Gulino *et al.* (17) produced a fragment composed of the four EF-hand sites within the α_{IIb} subunit and found that it contained two affinity classes for Ca^{2+}, which could also bind Mg^{2+} and Mn^{2+} and fibrinogen, a physiologic ligand for this integrin. These observations are generally consistent with results obtained from Ca^{2+} binding studies on the purified integrin (18) and synthetic peptides corresponding to the individual loops of each EF-hand (19). The EF-hand domains of the α_5 integrin also contain two affinity classes of ion binding sites and can bind fibronectin and RGD peptides (20). All four EF-hands were required for ligand binding, even though each pair of EF-hands was able to bind divalent ion.

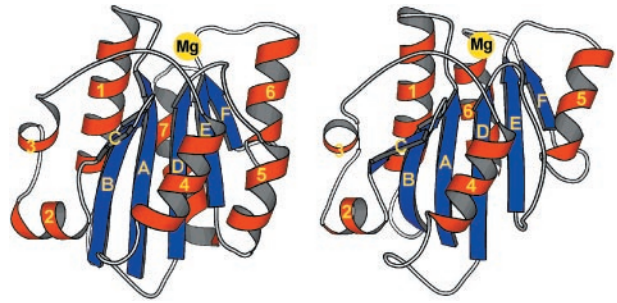

FIG. 1. **Structures of representative I domains in integrin α subunits.** The ribbon diagrams are derived from the crystal structures of the I domains of the α_M subunit (*left*) and the α_L subunit (*right*). These I domains display the typical α helical/β strand fold of I domains. The cation bound in the MIDAS motif appears on the upper face of the structures. These crystal structures for the α_M I domains were reported by Lee *et al.* (22) and by Qu and Leahy (23) for the α_L.

The second type of cation binding site in integrins is a metal ion-dependent adhesion site (MIDAS) motif. The first evidence for a unique cation binding motif came from mutagenesis studies of the I domain of the α_M subunit (21). Soon thereafter, the I domain of the α_M subunit and other integrin α subunits were crystallized (22–24). In Fig. 1, the crystal structures of two I domains are displayed with the MIDAS motif at their upper surface. Within the MIDAS motif, five separate residues coordinate the divalent ion. The first three are closely spaced within a D*X*S*X*S motif; the fourth is a threonine separated from the D*X*S*X*S in the primary structure by ~70 residues; and the fifth coordinating ligand is an aspartate about 100 residues downstream of the D*X*S*X*S. In the crystal structure, two of the α_MI domains were linked via a Mg^{2+} ion in the MIDAS motif, and a glutamate from one I domain donated a sixth coordinating ligand to the Mg^{2+} bound in an adjacent I domain. This quirk in the crystal structure provided evidence that metal ion bound to the MIDAS can ligand with carboxylates donated from another protein, consistent with the cation displacement model. Indeed, this finding has prompted the hypothesis that such a structure is a snapshot of I domain bound with "ligand" and led to the prevailing notion that integrins bind to their ligands by "grabbing an Asp" (25).

All integrin β subunits may contain an ion binding site homologous to a MIDAS motif. This proposition stems from early work showing that a naturally occurring mutation of Y119D in integrin $\alpha_{IIb}\beta_3$ led to a receptor with abnormal ligand and cation binding functions (26), and it was proposed that this residue was part of an EF-hand. In retrospect, this ion binding site is more likely to be a MIDAS motif with Asp-119 being the first residue of the D*X*S*X*S motif. Mutation of any of these putative ion-coordinating residues within the β_1, β_2, β_3, or β_5 subunits ablates ligand binding to integrins (*e.g.* Refs. 27 and 28). Although the D*X*S*X*S motif appears to ligand with metal, the residues that constitute the fourth and fifth coordinating ligands in the β subunit MIDAS remain unclear and controversial (27–29). The three-dimensional structure of a β subunit may be necessary for resolution. Also, there is no structural information to locate the I Ca^{2+} binding site that is found on most integrins. All of the ion binding sites that have been located, the EF-hand sites in the α subunits and the MIDAS motifs in the β subunits, appear to promote ion binding, *i.e.* are LC sites (15, 27, 30). One of the more tantalizing hypotheses regarding the location of this I site is that the ion binding site within the integrin β subunits can fold into either an EF-hand or a MIDAS domain. It may act as a MIDAS when Mn^{2+} or Mg^{2+} is present, but Ca^{2+} could induce an EF-hand conformation.

What Physiologic Role Do Ion Binding Sites Play in Regulating Integrins?—Despite the strong influence of divalent ions on cell adhesion and ligand binding to integrins, information on the actual physiologic role of the ion binding sites is limited. One area where there is clear physiologic relevance is the adhesion of bone-resorbing osteoclasts. The osteoclast adheres to the bone surface primarily through $\alpha_v\beta_3$ (31). Adhesion to the bone surface must be tightly regulated to prevent excessive resorption. Interestingly, as mineralized bone is resorbed, the concentration of free Ca^{2+} beneath the osteoclast becomes elevated and induces osteoclast detachment

and cessation of resorption. This effect may be mediated by the allosteric I Ca^{2+} binding site on $\alpha_v\beta_3$. Another situation where the levels of Ca^{2+} and Mg^{2+} fluctuate is in the fluid of healing wounds (32). During the initial healing process, the levels of Mg^{2+} increase, and the ratio of Mg^{2+}/Ca^{2+} elevates from the normal plasma ratio of 0.4:1 to approach 1:1. Because Mg^{2+} generally promotes cell adhesion and Ca^{2+} is generally inhibitory, this increase in ratio may be "promigratory," facilitating wound closure. Finally, the ion binding sites also may play a role in integrin activation. Mn^{2+} increases the apparent affinity/avidity of multiple integrins for their ligands. Mn^{2+} binds to MIDAS motifs, and two conformations of the MIDAS can be distinguished by the manner in which the bound metal is coordinated (33, 34). Thus, Mn^{2+} could induce structural rearrangements in the I domains, which result in activation. An equally attractive hypothesis suggests that activation is regulated by the binding of Ca^{2+} to the allosteric I site. Occupation of this site by Ca^{2+} would maintain the integrin in a resting state.

The β_2 Integrins, Representative I Domain-containing Integrins

The β_2 subfamily consists of four different integrin receptors, $\alpha_M\beta_2$ (CD11b/CD18, Mac-1, CR3, Mo-1), $\alpha_L\beta_2$ (CD11a/CD18, LFA-1), $\alpha_X\beta_2$ (CD11c/CD18), and $\alpha_D\beta_2$ (CD11d/CD18). These leukocyte integrins are involved in virtually every aspect of leukocyte function, including the immune response, adhesion to and transmigration through the endothelium, phagocytosis of pathogens, and leukocyte activation. The importance of the β_2 integrins is underscored by the susceptibility of patients lacking these integrins to severe infections (see Ref. 35, and references therein). However, excessive activation of $\alpha_L\beta_2$ and $\alpha_M\beta_2$ contributes to sustained inflammation, reperfusion injury, and tissue damage. Recently, it has become possible to gauge the function of individual β_2 integrin receptors in mice rendered selectively deficient in $\alpha_M\beta_2$ and $\alpha_L\beta_2$. The leukocyte integrins are the subject of a separate review in this miniseries. The discussion herein focuses primarily on their I domains and their role in ligand binding.

The α subunits of all β_2 integrins contain an *inserted* region of ~200 amino acids, termed the I or A domain. Highly conserved I domains are found in several other integrin α subunits and other proteins, such as certain coagulation and complement proteins. I domains mediate protein-protein interactions, and in integrins, they are integrally involved in the binding of protein ligands (14, 22, 36). I domains, including those in integrin α subunits, fold independently, can be expressed as recombinant fragments, and can bind ligands. Even though I domains are highly homologous to each other, they are highly selective for particular sets of ligands. At the same time, a single I domain can recognize multiple and structurally unrelated ligands (see ligand repertoire of $\alpha_M\beta_2$ in Table I). Thus, it is the individual amino acid differences within the highly conserved structural fold of the I domains that impart ligand specificity.

The crystal structures of several integrin and non-integrin I domains have now been solved (*e.g.* Refs. 22–24). Each consists of five parallel β sheets surrounded by 5–6 α helices, which are interconnected by flexible loops (see Fig. 1). The I domains of the β_2 integrins contain the previously discussed cation binding MIDAS motif. In the I domains of $\alpha_M\beta_2$ and $\alpha_L\beta_2$ shown, the binding interface for several ligands has been mapped to the upper face, in close proximity to the bound cation (34, 37). Mutations in other faces of the I domains or outside the I domains can exert allosteric effects, either activating or inhibitory, on ligand binding (34, 38, 39).

Although the I domains dominate the ligand binding functions of their integrins, other regions of the α subunits do influence ligand recognition. As examples, in $\alpha_M\beta_2$ a mAb (OKM1) recognizing an epitope outside the I domain but in the α_M subunit inhibits ligand binding (40); and the EF-hand regions in $\alpha_L\beta_2$ and $\alpha_2\beta_1$, integrins with I domains in their α subunits, contribute to ligand recognition (41, 42). The α_M subunit, and perhaps other α subunits, contains a lectin-like domain, which is involved in engagement of non-protein ligands, and occupancy may modulate the function of the I domain (43). The role of the β subunit in ligand binding to the leukocyte integrins is complicated. Though the importance of the β subunit in ligand recognition has been demonstrated using blocking mAbs and site-directed mutagenesis (*e.g.* Ref. 44), its direct involvement in ligand contact has yet to be established. Mutations in the β_2 subunit, which abolish ligand binding, may exert a *dominant-negative* effect on ligand binding (45).

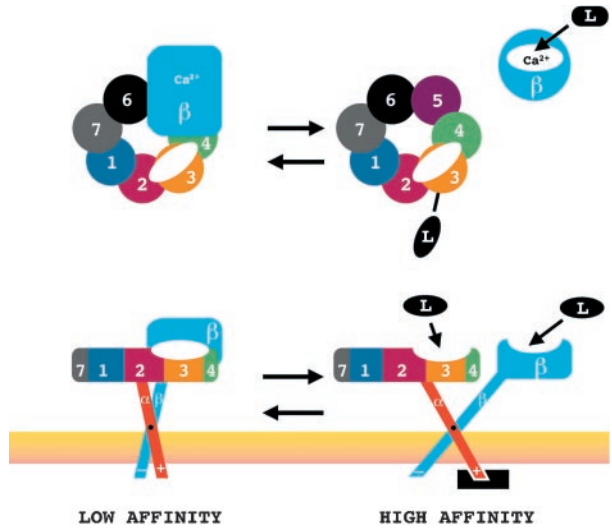

FIG. 2. **Top (*upper*) and side (*lower*) views of a model of the ligand binding sites on $\alpha_{IIb}\beta_3$.** The *numbers* refer to the seven repeats in the α_{IIb} subunit, and the Ca^{2+} shown is bound to the MIDAS motif in the β_3 subunit. In the low affinity state, receptor conformation does not favor binding of macromolecular ligands (*L*). Activation alters the spatial relationship of the two subunits and the conformation of individual binding domains to allow high affinity ligand interaction with multiple contact sites.

Ligand Binding to Integrins without I Domains: Paradigms from $\alpha_{IIb}\beta_3$

The central role that platelet adhesive reactions play in hemostasis has focused significant efforts on defining $\alpha_{IIb}\beta_3$ recognition sequences within its ligands and the ligand contact points within the receptor. Many of the insights gained from these studies extrapolate to the β_1 integrins because several of the β_1 and both β_3 integrins share a RGD recognition specificity. A second recognition specificity of particular importance to the function of $\alpha_{IIb}\beta_3$ is the C terminus of the fibrinogen γ chain (46). Each sequence contains an aspartic acid that is critical for recognition, perhaps through an interaction with receptor-bound cation (26). The two recognition peptides inhibit the binding of each other to $\alpha_{IIb}\beta_3$ but may bind to separate but allosterically linked sites (30, 47).

Substantial data have accumulated regarding the location of potential ligand contact sites within $\alpha_{IIb}\beta_3$. Mapping the epitopes of inhibitory mAbs, enzymatic digestion of native $\alpha_{IIb}\beta_3$, and the expression of soluble, recombinant forms of $\alpha_{IIb}\beta_3$ have indicated that the minimal ligand binding fragment contains the N-terminal half of each subunit (48). The specificity of $\alpha_{IIb}\beta_3$ *versus* $\alpha_v\beta_3$ for ligands was mapped to the N-terminal 334 residues of α_{IIb} (49). Cross-linking studies have demonstrated the existence of ligand contact points in the N-terminal portions of α_{IIb} (50) and β_3 (51, 52), and it is likely that binding of macromolecular ligands to $\alpha_{IIb}\beta_3$ involves multiple contacts in each subunit. Essential residues identified to date can be grouped into two major regions: 1) the highly conserved segment, residues 95–400 of the 762 amino acids in β_3; and 2) the seven N-terminal repeats of α_{IIb}.

Two distinct, discontinuous regions within β_3 clearly contribute to the ligand binding function of the receptor. RGD peptides cross-link to a region of β_3 defined by Asp-109 to Glu-171 (52). An overlapping region of β_3 (Glu-65 to Glu-220) was identified by cross-linking of RGD peptides to $\alpha_V\beta_3$ (53). As noted previously, a naturally occurring point mutation in this region, D119Y, results in complete loss of ligand binding function (26), as do mutations at residues in this vicinity (54). The second potential ligand interactive site in β_3 is defined by residues Ser-211 to Gly-222. Peptides corresponding to this sequence and antibodies directed against these peptides inhibit fibrinogen binding (55) as do natural mutations at β_3 Arg-214 (56, 57). The importance of these two discon-

tinuous regions of β_3 is likely because of their participation in the formation of a MIDAS motif (21, 22) although, as noted above, it remains uncertain as to whether this region of β_3 adopts an I domain fold (27, 29). Whereas the two discontinuous segments of β_3 are involved in cation binding, they also may provide direct contact sites for ligand. Peptides and recombinant fragments of β_3 bind fibrinogen and γ-chain and RGD peptides (47, 58).

Studies of the ligand contacts in α_{IIb} have established a role for its seven N-terminal repeats. As noted above, a recombinant fragment of α_{IIb} containing the four EF-hand-like sequences binds fibrinogen in a Ca^{2+}- and RGD-dependent manner (17). This region of α_{IIb} has been further implicated in ligand binding because ligand mimetic peptides cross-link within the second cation binding repeat (50). Peptides from this region inhibit fibrinogen binding to $\alpha_{IIb}\beta_3$ and directly bind fibrinogen in a Ca^{2+}-dependent manner (59, 60). Together, these data suggest a functional importance of the α_{IIb} cation binding domains in ligand interactions. Paradoxically, a minimized form of αIIb, Leu-1 to Gly-233, which does not contain any of the divalent cation repeats, associated with β_3 and recognized ligand (61).

Several attempts have been made at developing a structural model of integrin α subunits. A recent model proposes that the seven N-terminal repeats adopt the fold of a β-propeller domain (62). These domains contain seven four-stranded β-sheets, or "blades," arranged in a torus around a pseudosymmetry axis. Enzymes with known β-propeller folds have their active sites at the top of the β-propeller, typically where adjacent loops run in opposite directions. Consistent with this hypothesis, the α_{IIb} residues, Gly-184 to Gly-193, located in one loop, and α_{IIb} Asp-224, located in a second loop, both predicted to be at the top of the β-propeller, have been implicated in ligand binding by site-directed mutagenesis (63, 64). The identification of residues critical for ligand binding to $\alpha_4\beta_1$ and $\alpha_5\beta_1$ further supports the β-propeller model (65, 66). Nevertheless, this model appears to contradict the data implicating the direct involvement of the α_{IIb} and α_5 cation binding motifs in ligand binding. The model places these motifs on the lower surface of the propeller, spatially distant from the putative ligand contact points on the upper surface of the propeller.

The data discussed above confirm that the ligand binding pocket consists of portions of both the α_{IIb} and β_3 subunits and is consistent with the concept that binding of macromolecular ligands involves multiple contact points in the receptor. An emerging model (Fig. 2) is that $\alpha_{IIb}\beta_3$ has at least two distinct ligand binding domains that are intimately linked and allosterically controlled. $\alpha_{IIb}\beta_3$ exhibits significant conformational changes during activation and ligand binding. Therefore, it will be important to understand how these changes influence the individual ligand binding domains as well as how these changes affect the manner in which the α_{IIb} and β_3 subunits fold against each other to establish a structural basis for ligand binding to $\alpha_{IIb}\beta_3$.

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