Structural Aspects of Integrins

8

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Abstract

Structural studies on integrins have recently made great strides in recent years. Crystal structures of the complete extracellular fragments of three integrins in open and closed conformations, 6 α-I domains in complex with ligands, and at least 20 intracellular proteins in complex with cytosolic tails have been obtained; and several transmembrane and cytosolic complexes have been determined by NMR. High resolution EM studies complement these atomic resolution techniques by studying the integrin in different activation states. Although we still have only a few experimental examples among integrin family members, the high level of sequence homology between integrins means that reliable models can be built for the other members of the integrin family. These structures make sense of a lot of preceding biochemical, biophysical and mutagenesis studies, and generate many new testable hypotheses of integrin function. This chapter emphasizes new structural insights applicable to all integrins, with an emphasis on those integrins that contain an α-I domain. The structural data reinforce the notion of the integrin as a molecule in dynamic equilibrium at the cell surface, regulated by binding both to extracellular and intracellular ligands.

Keywords

Integrins · Structure · Mechanism · Allostery

8.1 Overall Structure

Integrins are $\alpha\beta$ heterodimers, consisting of a head domain from which emerge two legs, one from each subunit, ending in a pair of single-pass transmembrane helices and short cytoplasmic tails,

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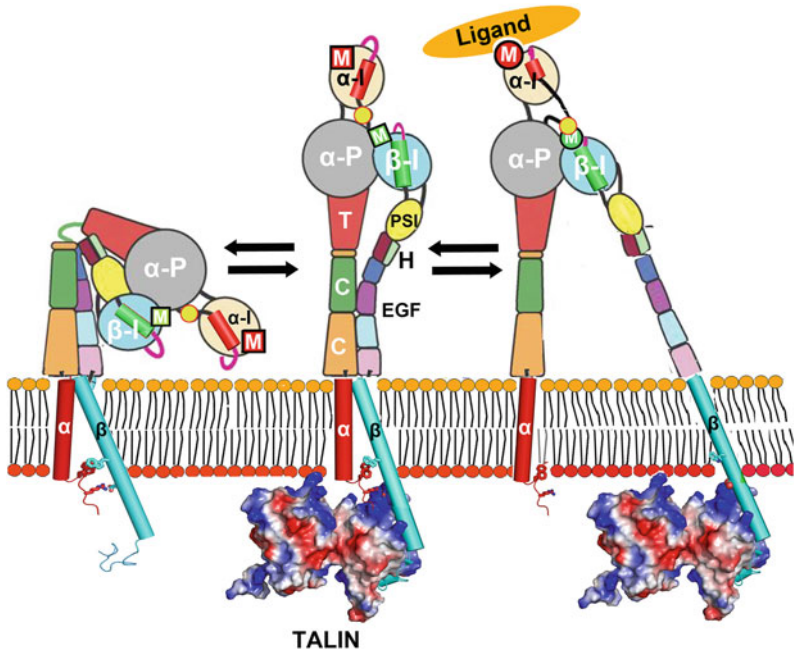

Fig. 8.1 Cartoon of the αXβ2 structure derived by crystallography and EM studies. At *left*, the bent, low affinity integrin stabilized by bonds between the head, legs and cytoplasmic tails. At *center*, an unknown trigger causes the integrin to "stand up", while maintaining most of its low affinity bonds. At *right*, binding of activated talin and/or binding of an extracellular ligand, trigger an open, high affinity form of the integrin, with TM helices separated

except for α6β4 (Fig. 8.1). The integrin "head" comprises a seven-bladed propeller from the α-subunit that makes an intimate contact with the β-I domain. Nine α-subunits (α1, α2, αl0, α11, αD, αE, αL, αM and αX) contain an additional domain, the α-I domain, that is inserted between two loops on the upper surface of the propeller, where it plays a central role in ligand binding [27, 41, 58]. The α-I domain contains an invariant ligand binding site called MIDAS, for Metal Ion-Dependent Adhesion Site [34], in which a metal ion is coordinated by three loops from the I domain, and a glutamic or aspartic acid from the ligand completes an octahedral coordination sphere around the metal. In those integrins that lack an α-I domain, the β-I domain and propeller form the major ligand recognition sites; in the α-I domain integrins, the β-I domain plays a regulatory role.

In the absence of ligand, bonds between the legs, tails and head are believed to hold the head in an "inactive" or "resting" conformation that has low affinity for ligand [20, 56, 61]. Recent structural data suggest that integrins possess three global conformations (see Fig. 8.1): a bent conformation in which the head adopts a "closed", low affinity conformation and the cytoplasmic tails form an inhibitory complex; an extended conformation of the head that retains its low ligand affinity; and a high affinity form in which the legs and tails separate, and the "hybrid" domain, which is part of the head, swings away from the β-I domain, propeller and α-I domain, promoting conformational changes that create high affinity binding sites on both the head and tail [54]. During "outside-in" signaling, the head binds to ECM proteins or counter-receptors on other cells, triggering conformational changes that propagate down the "legs" and through the plasma membrane, leading to a reorganization of the C-terminal tails that allows them to bind intracellular proteins [46]. During "inside-out" signaling, cytosolic proteins bind and sequester one or both of the cytoplasmic tails, triggering conformational changes in the head that promote a high affinity "active" integrin [19, 60], in which the integrin "stands up".

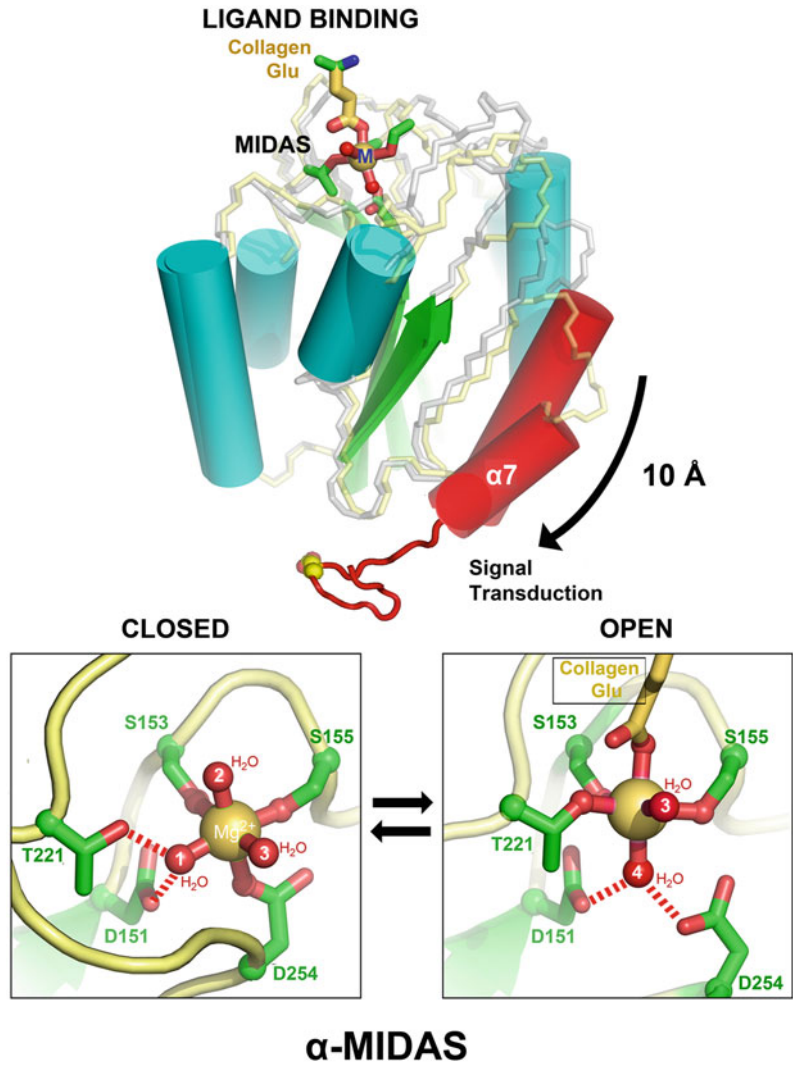

Fig. 8.2 Two conformations of the α-I domain. *Upper panel* conformational changes in the α2-I domain on binding collagen. *Lower panel* Conformational changes in the α-MIDAS motif upon binding ligand. At *left*, the "closed" conformation observed in the absence of ligand; at *right*, the "open" conformation seen when ligand is bound. These changes are mechanically linked to the tertiary changes in the domain. It is very likely that all α-I domains undergo the same switch

8.2 The α-I Domain

The first crystal structure of an α-I domain revealed a compact domain comprising a central mostly parallel β-sheet surrounded on both sides by amphipathic α-helices [34] (Fig. 8.2). Subsequent crystal structures of recombinant αL, α1 and α2 I domains display the same three-dimensional fold, as expected given their reasonable sequence similarity [15, 43, 45]. The MIDAS motif lies at the C-terminal end of the central β-sheet, with three loops contributing sidechains that coordinate the metal ion (Fig. 8.2 Lower panel). The metal-coordinating MIDAS residues are invariant among α-I domains, and mutagenesis of any of these residues abrogates ligand binding. Surface-exposed sidechains surrounding the MIDAS motif are more variable; they provide additional ligand contact points and hence ligand specificity [26, 41, 52].

The structures of 6 ligand-bound α-I domains have now been determined. The first was the α2-I domain bound to a collagen-like triple helix [17]. More recently, the structures of the αL-I domain in complex with homologous fragments

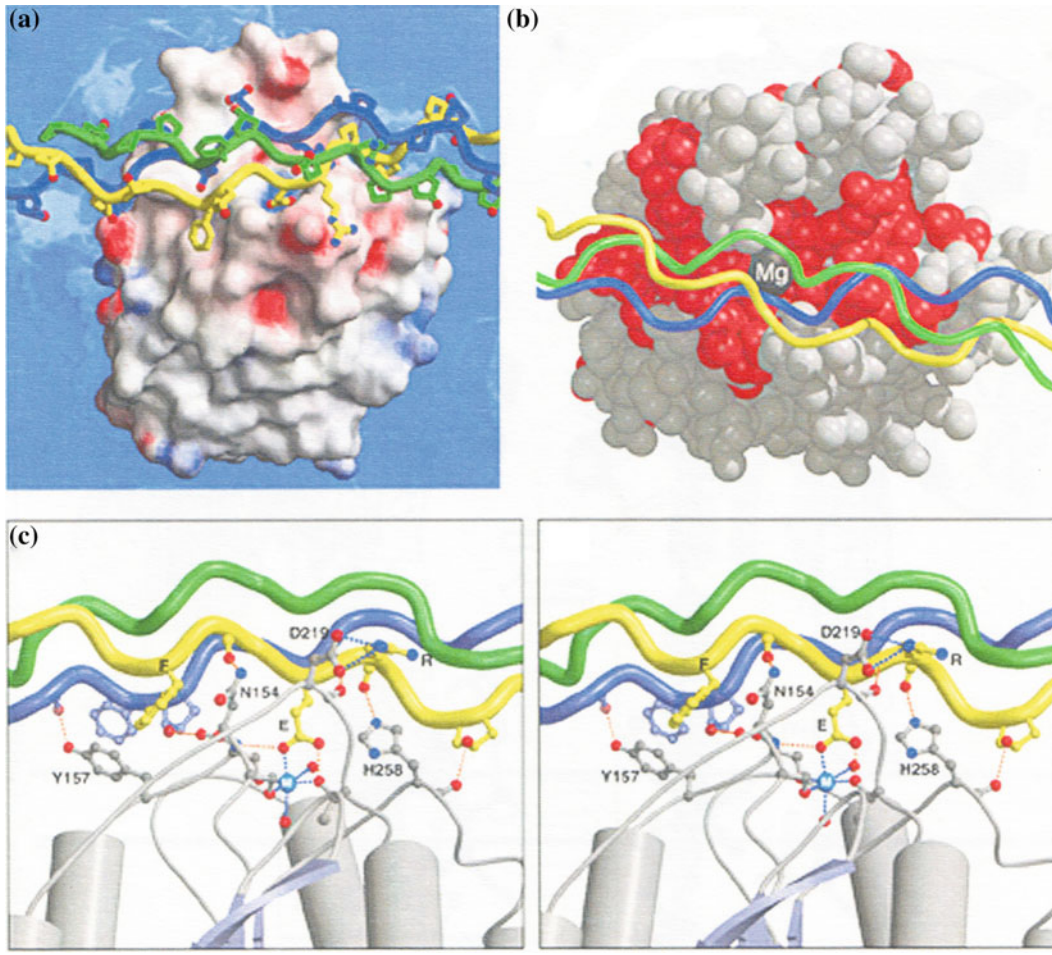

Fig. 8.3 Collagen binding to the α2-I domain. **a** Surface model of the α2-I domain colored by surface charge (*red* = negative, *blue* = positive) with a triple helical fragment of collagen bound. **b** Space-filling model of the complex (rotated about a horizontal axis compared with **a**), showing residues (in *red*) that are invariant in the collagen-binding integrins, α1β1, α2β1 and α10β1. The strong conservation of the binding surface suggests that these integrins will engage collagen in the same fashion. **c** Stereo close-up image of the α2-I:collagen complex

of ICAM-1 [51], ICAM-3 [53] and ICAM-5 [67] have been determined. Recently, the first authentic complex of an αM-I domain bound to ligand (the C3d domain of complement C3) [2] validates the earlier structure of the αM I domain bound to a "ligand-mimetic" crystal contact [33, 34]. They all demonstrate that ligand binding triggers a profound conformational switch in the α-I domain that underlies affinity regulation and signal transduction. The conformational switch is essentially identical in all these examples, strongly suggesting that all α-I domains will undergo the same switch.

8.3 Structural Determinants of Collagen Binding

Recombinant α2-I domain was crystallized as a complex with a homotrimer of a 21-mer peptide containing a critical GFOGER (where O is hydroxyproline) motif [17, 30]. The peptide closely resembles the structure of uncomplexed collagen-like peptides [16], and has the properties of a folded protein domain (i.e., stable secondary and tertiary structure). Three loops on the upper surface of the α2-I domain that comprise

the MIDAS motif also engage the collagen, with a collagen glutamate completing the coordination sphere of the metal (Fig. 8.3). The critical roles of both the MIDAS and surrounding residues have been confirmed by mutagenesis [52].

The buried surface area on complex formation ($\sim$1,200 A^2) is at the lower limit of known protein-protein interfaces (the value is almost identical for the αL-I:ICAM complex), especially given the fact that some of the binding energy must be expended in switching the conformation of the I domain from closed to open. The quite reasonable affinity of the interaction (Kd = 35–90 nM) [24] reflects the unusually strong bonds formed by the glutamate-metal-I domain bridge, which has been estimated to contribute $\sim$5 kcal/mol. This bridge is indeed critical, since the conservative substitution of collagen Glu to Asp in the GFOGER motif eliminates binding [31], presumably because the aspartic acid is too short to reach down from the rigid collagen triple helix to bind to the partly buried metal ion. However, in the case of the αM-C3d interaction, Asp is the preferred residue, perhaps because it lies on a flexible loop at the end of a helical segment [2].

The MIDAS motif and much of the collagen-binding surface are strictly invariant among the collagen-binding I domain integrins (αl, α2, α10 and α11), suggesting that these integrins will all engage collagen in a similar fashion, with a strict requirement for glutamate in the collagen motif. The periphery of the binding surface is more variable, however (Fig. 8.3b), which would explain their collagen type preferences. The recent structure of the α1-I domain bound to collagen containing the closely related motif, GLOGEN, confirms this [11]. In addition, a gain-of-function point mutation in the α2-I domain (i.e. one that favors the open conformation) [10] displays relaxed specificity and alternate binding modes to the GFOGER motif. Given the special nature of collagen (see Chap. 3 by Zutter and Santoro), this observation may point to profound consequences for collagen recognition by activated cells.

8.4 The Integrin α-I Domain and the von Willebrand Factor (vWF) A Domain: A Caveat

The integrin α-I domain is generally categorized as a member of the vWF-A domain superfamily, based on sequence similarity and a highly conserved overall 3-dimensional structure. However, since MIDAS- and non-MIDAS containing vWFA-domains have distinct ligand-binding and allosteric properties, this author believes that much confusion could be avoided if the family was reclassified into two sub-families: Two examples illustrate my point. First, the eponymous vWF-A1 and vWF-A3 domains lack at least one of the acidic residues of authentic MIDAS motifs, and so do not bind metal; moreover, they bind ligands via distinct surfaces, and conformational changes are not induced [21]. In fact, vWF-A3, like integrin α2β1, binds triple-helical collagen, but it utilizes a different surface (one side of its β-sheet) [7]. Second, the "vWF-A domain" of Factor B *does* contain a functional MIDAS motif, and binds an acidic moiety in its ligand, complement iC3b, in the canonical integrin fashion; in this case, the metal ion engages the C-terminal carboxylate iC3b, triggering integrin-like conformational changes [18], suggesting that it should be classed as an I domain. Indeed, a genome-wide collection of vWF-A domains has been compiled [62] and have been these subdivided into I-like and A-like domains based on the conservation of key MIDAS residues.

8.5 Conformational Changes in the α-I Domain

Ligand binding alters the conformation of α-I domains in the same way in the three cases studied thus far (α2, αM and αL), as well as in a subset of "vWF-A domain" (as noted above) and the matrix receptor, TEM8 (in complex with pathogen; see below). Binding of an acidic residue to the α-MIDAS causes a switch in in Mg^{2+}

coordination in which a direct bond to a MIDAS threonine is gained while a direct bond to an aspartic acid is lost (Fig. 8.2b, c). These subtle changes in metal coordination are linked to extensive secondary and tertiary changes that create a complementary surface for binding ligand and generate a 10 Å downward movement of the C-terminal helix, α7. The helix movement links the change in the upper ligand-binding surface to the lower surface of the domain. The shift of the helix α7 is highly significant in the context of the whole integrin, since the helix is packed against the propeller and β-I domain (see Sect. 8.8).

The close similarity between the structural changes seen in the three α-I domains and a subset of A domains suggests that there are just two principal conformations for I domains, "open" and "closed" (Fig. 8.2). The "open" conformation is seen in the presence of ligand or ligand mimetic, while the "closed" conformation is seen in the absence of ligand. It therefore appears to be the formation of a strong ligand-metal bond, requiring a change in metal coordination, that triggers the conformational switch. Springer's group has engineered disulfide-linked αL-I domains with intermediate affinity and packing of the C-terminal helix, and suggested the existence of an intermediate state [51]. However, in these structures the MIDAS motif exists in only two conformations, and it remains to be seen whether the intermediate conformation has biological relevance or is an artifact of the engineered disulfide. It should be noted that it is not necessary to invoke an intermediate tertiary conformation in order to explain an intermediate affinity. In principle, a shift in the position of the equilibrium between two states is sufficient [38].

Various studies have now been published in support of the hypothesis that the open and closed conformations of the α-I domain equate with high and low affinity states. Thus, mutants of the αM-I domain that are predicted to destabilize the closed conformation and favor the open conformation increase the affinity for the ligand iC3b [41]. The epitope for an antibody that binds only to the high affinity form of the αMβ2 integrin maps to a region that undergoes extensive conformational changes between the closed and open forms [35].

Disulfide engineering studies on recombinant I domains and full-length integrins, which lock the domain either into the open or closed state, also support the hypothesis [39, 49, 50]. So does the structure of the αL-I domain in complex with the inhibitor lovastatin [25], which reveals allosteric inhibition by binding between the β-sheet and the C-terminal helix, preventing the helical shift.

It should also be appreciated that pathogens often utilize integrin α-I domains for cell entry, and there is evidence that many bind across the MIDAS motif. However, in general they bind preferentially to the (default) closed conformation, sometimes involving direct bonds to the MIDAS, but they do not induce conformational changes [4]. One counter-example is anthrax toxin, which utilizes a glutamate to engage the *bona fide* MIDAS motif of the "vWF A domain" of the collagen receptor, TEM8, in its open conformation [6]. There is also one clear example of gene transfer, in which the Gram-positive pathogen, *Streptococcus pneumoniae*, has an α-I domain inserted into the tip of its pilus, perhaps to act as a shear stress-activated adhesin for attachment to host cells [23, 36].

8.6 The β-I Domain and the Integrin Headpiece

The existence of a β-I domain was initially predicted based on the conserved and critical MIDAS-like sequence, DxSxS, and hydropathy plot comparisons with the α-I domain [34]; and later from more sophisticated sequence analysis [59]. The structure of the β3-I domain, contained within the αVβ3 crystal structure [64], confirmed that the basic fold and topology are very similar to the α-I domain, albeit with many large insertion/loops between β-strands, which had confounded conventional sequence alignment algorithms.

In contrast to α-I, the β-I domain is not folded independently, but packs rigidly against the α-subunit propeller, with the major ligand

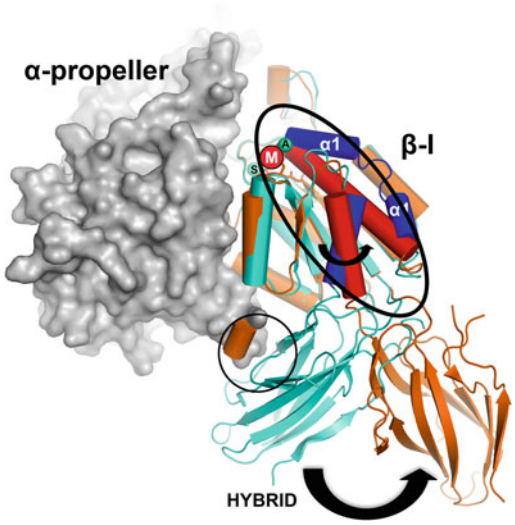

Fig. 8.4 Tertiary and quaternary changes triggered by ligand binding in integrins that lack an α-I domain. Ligand binding to the *β*-MIDAS motif (M) causes a shift of helix α1, which generates a rotation of α7 helix (*black arrow* within region *circled* in *black*) and a loosening of the contacts between the *β*-hybrid domain and the propeller. The *β*-hybrid domain is then free to swing by as much as 60° away from the α-propeller

recognition elements lying at the interface (see Fig. 8.4) [42, 64]. The *β*-MIDAS is similar to the α-MIDAS, except that the α-MIDAS threonine is replaced by glutamate. This difference likely explains the different cation specificities—in α-MIDAS, the smaller Mg^{2+} ion favors ligands lacking a formal charge; while in *β*-MIDAS, the larger Ca^{2+} ion favors multiple acidic ligands. The structure of α*V*β3 in complex with an RGD-style peptide shows that the Asp sidechain completes the coordination sphere of the MIDAS metal ion [65], as predicted. There are also further metal-binding sites adjacent to the *β*-MIDAS (the "ADMIDAS" and "SyMBS") that play important structural and possibly regulatory roles in ligand-binding and regulation [68].

Although Xiong et al. initially proposed the opposite, the conformation of the *β*-I domain in their unliganded crystal structure [64] corresponds to the closed conformation of the α-I domain. Soaking of RGD ligand into preformed crystals induced small changes within the *β*-I domain, but these were not propagated to the rest of the headpiece; i.e., they were frustrated by the constraints of the closed quaternary structure [37]. This situation is typical in crystallography: either the ligand binds and induces small changes constrained by the lattice, or it induces large changes that destroy the lattice.

However, Springer's group has recently discovered a rare exception to this rule, and report a crystal form of the αIIb*β*3 headpiece with large solvent channels in which the lattice tolerates (and/or adjusts to) a switch from the closed to the open conformation, involving an outward swing of the hybrid domain by ~40°. Preformed crystals were simply soaked with different concentrations/durations of an RGD ligand mimetic and different Ca^{2+}/Mg^{2+} ratios [69] (Fig. 8.4). This remarkable observations settles many questions with regard to head-opening, although the crystal structure of the headpiece in complex with a non-peptidic ligand is still lacking.

8.7 Quaternary Regulation in Integrins Lacking an α-I Domain

Takagi et al. [57] showed that the inactive (resting) form of the integrin α*V*β3, observed in physiological concentrations of Ca^{2+} and Mg^{2+}, is largely bent, and closely resembles the crystal structure, in which the C-termini of both chains are closely apposed. Based on the one case studied of an α-I domain integrin, the α*X*β2 ectodomain, it also adopts a similar (although distinct) bent default conformation [63]. Other integrins tested had a lower propensity to adopt the bent conformation; however, the experiments were performed with extracellular heterodimers truncated near the plasma membrane, so that they lacked the transmembrane helices and cytoplasmic tails that are known to contribute critically to the stability of the inactive conformation. By engineering a disulfide link between the α-subunit propeller and the EGF4 domain of the *β*-subunit (which are 4 Å apart in the bent (crystal) structure, but would be very far apart in the "standing-up" conformation), Takagi et al. further showed that integrin expressed on the cell surface was in a

low affinity state and could only be activated under reducing conditions.

The current model for integrins invokes a minimum of three distinct states: (i) bent, low affinity; (ii) standing-up, legs together, low affinity; and (iii) standing-up, legs apart, high affinity (see Fig. 8.1). The position of equilibrium depends on the concentrations and activation status of extracellular and intracellular ligands, as well as divalent cations. At the heart of the switch is an outward swing of the β-hybrid domain with respect to the β-I domain, by as much as 60° (Fig. 8.4). In αIIbβ3, the primary response to extracellular ligand binding is a concerted reorganization of the N-terminal helix (attached directly to the β-MIDAS) and the adjacent C-terminal helix. Rather than translate downward (as in the case of the α-I domain), the principle motion of α7 is a rotation about an axis close to the β-MIDAS, which is linked to the rotation of the β-hybrid domain. Thus, although some details may differ, the data support the prediction that the trigger for the integrin switch is similar in integrins that contain or lack and α-I domain: i.e., a subtle change in metal coordination at the MIDAS motif is linked to a reorganization of the I domain architecture that leads to quaternary changes toward an open, high-affinity state [33].

As noted above, these experiments were performed with truncated integrins and small peptide ligands. The nature of the trigger in the integrin head seems secure, but it remains to be seen how the quaternary changes are promulgated across the plasma membrane. Recent studies have shown that full-length integrin can be reconstituted into lipid nanodiscs and visualized by high resolution Electron Microscopy [12], so we should soon have an answer.

8.8 Quaternary Changes in Integrins Containing an α-I Domain

As noted above, in integrins that lack an α-I domain, the β-I domain and α-subunit propeller are the major recognition elements [42]. However, in integrins that contain an α-I domain, the β-I and α-propeller do not play direct roles in ligand recognition; instead they play important regulatory roles. This concept initially caused some confusion: thus, mutation of the β-MIDAS motif led to loss of iC3b binding to αMβ2 [3] which was initially interpreted as evidence for a direct role for the β-I domain in ligand; it now seems clear, however, that the mutation works allosterically, by preventing conformational changes in the α-I domain.

How does the quaternary organization of the integrin regulate the affinity of the α-I domain? We know that regulation occurs allosterically (rather than by steric masking of the binding site), since the α-I domain is a major antibody epitope. Hypotheses focused on the loss-of-function effect of mutating a Glu residue within a conserved ΦEGT motif (where Φ is any hydrophobic residue) at the end of the α-I domain C-terminal (α7) helix [1, 22, 66]; and it was suggested that the Glu could act as an intradimer ligand by completing the coordination sphere of the β-MIDAS motif. The first crystal structures of the αXβ2 headpiece (from Xie et al. [63]) were inconclusive: they showed the α-I domain in the closed conformation, but rather loosely attached to the rest of the headpiece. However, a recent structure of the αXβ2 ectodomain displays an activated α-I domain by virtue of a fortuitous crystal contact [47]. Although the rest of the $\alpha\beta$ headpiece is in the closed conformation, the predicted "internal ligand", Glu318, is observed coordinating the β-MIDAS motif with only minor compensatory movements in the β-I domain (Fig. 8.5). By contrast, the α-I domain adopts a fully "open" conformation, with the MIDAS threonine directly coordinating the metal and (what appears to be) a chloride ion completing the coordination sphere. The first half of the α-I domain α7 helix has shifted by $\sim$10 Å, as expected, but the remainder is unwound, thereby switching the orientation with respect to the headpiece. Thus, the crystalline environment seems to have created a hybrid molecule with a fully active α-I domain in the context of an inactive headpiece. It is possible that such a

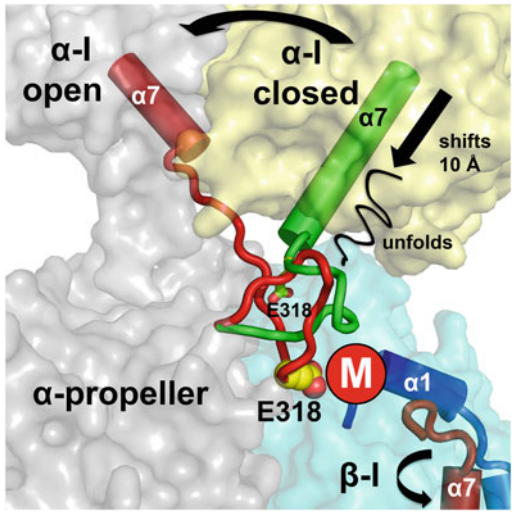

Fig. 8.5 Close-up comparison of the α-I domain-containing integrin head of αXβ2. In the open conformation, Glu318 acts as an internal ligand to the β-MIDAS that generates a 10 Å shift in the first half of helix α7 of the αI domain, while the second half of the helix unwinds, leading to a 30–40° rotation of the α-I domain about the propeller and β-I domain. The open α-I domain is stabilized by a crystal contact, and the the β-I domain remains principally in the closed conformation

hybrid state could exist in vivo, providing long-range, flexible and rapid (non-equilibrium) responses to the presence of ligand and/or mechanical stress; with a slow switch to the overall open (equilibrium) conformation occurring if the signal persisted.

8.9 Transmembrane (TM) and Tail Interactions

There are abundant biochemical and genetic data supporting the notion that interactions between integrin α- and β-TM helices and cytosolic tails help to hold the resting integrin in a low affinity conformation. In a classic study by Ginsberg and colleagues, a salt bridge between αIIb Arg995 and β3 Asp723 was shown to be necessary and sufficient to hold the integrin in its resting state [20]. A definitive structure of the αIIbβ3 tails in bicelles [32] reveals a remarkably stable conformation, in which the two helices pack closely together at the extracellular end; at the intracellular end they diverge, but the void is effectively filled by a highly conserved aromatic triplet that breaks the α-subunit helix and turns inward (Fig. 8.6). Isolated α and β subunit helices studies in bicelles show a remarkably well-conserved structure: the α-subunit is always orthogonal to the membrane while the β-subunit helix is always tilted. Recently, Ginsberg has shown that Lys 716β, whose Cα is buried in the membrane, can "snorkel" to the hydrophilic headgroups by extension of the Lys sidechain, and moreover that this residue is essential for maintaining the tilted helix and TM signaling [28, 55]. Recent studies on α-I domain integrins have yielded consistent results for isolated TM regions, and structures of αβ complexes are in progress [13]. The switch to the "open" conformation may entail a simple separation of the tails, which maintain their structural integrity and reassemble rapidly when the integrin returns to the low affinity state.

The penultimate question is how cytosolic proteins interact with the cytoplasmic tails of integrins, and the number of structural examples of protein domains bound to tail peptides (mostly β, but some α) has grown rapidly in recent years (see Table 8.1). It is clear that some proteins bind strongly enough to the β-tail to promote integrin activation. Talin was the first such molecule to be thus characterized, and remains the central player [8], although the number of additional contributors, such as kindlin and filamin [9], is growing fast. A model of talin activation is presented in Fig. 8.6. Talin sequesters the β-tail, breaking the critical R995α-D723β bond. It is also clear that phosphorylation of the β-tails provides a rapid means of switching between binding partners, and thus between cell migration and adhesion [14, 40, 44].

The final question is how the cytosolic activators generate force across the membrane. In the case of talin, recent work by Ginsberg's group suggests that dissociation of the tails, which have flexible linkages to the extracellular domains, is sufficient [29]. Key to this process is talin's ability to bind simultaneously to the integrin β-tail and membrane (Fig. 8.6), the latter providing a pivot point to force the two helices apart.

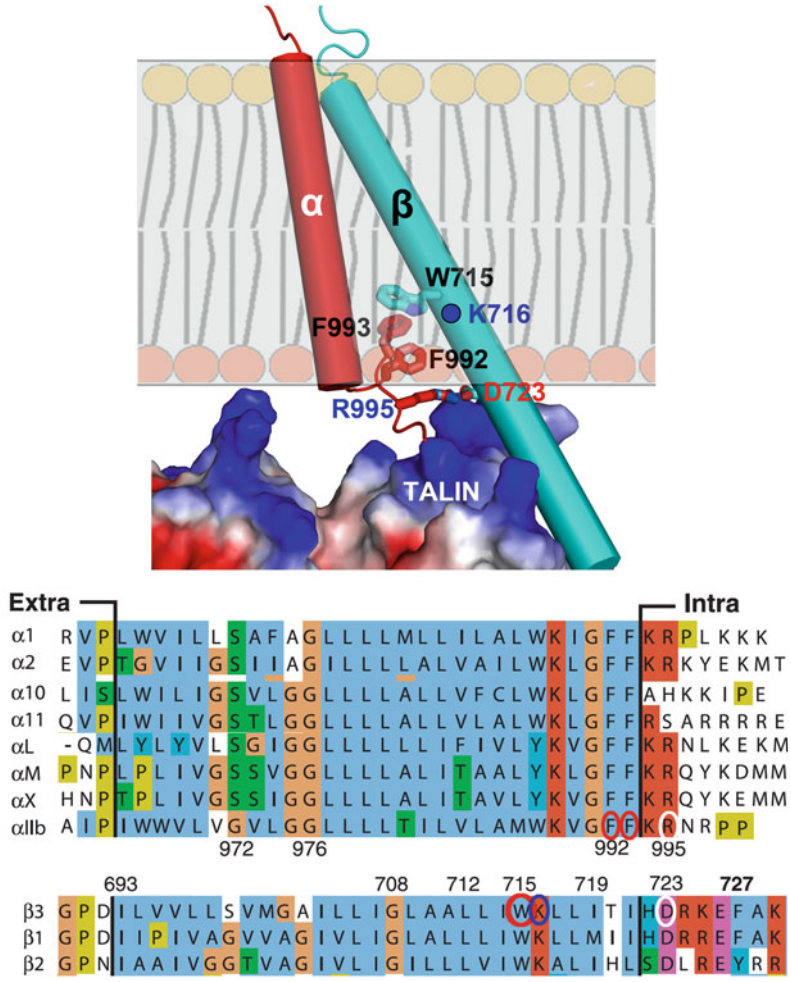

Fig. 8.6 Integrin-tail interactions. *Upper panel* Atomic interactions between the αIIb and β3 tails as revealed by NMR, melded to the complex of Talin2 and β1D tail. *Lower panel* Aligned sequences of α and β TM segments. Important residues in αIIbβ3 (and conserved in α-I domain integrins) are circled. See text for details

8.10 Perspectives

Structural and structure-function studies have revealed many of the major paradigms of integrin allostery underlying affinity regulation and bi-directional signal transduction. Notably lacking is the structure of an intact integrin bound to a physiological ligand in a membrane environment that would reveal the true "active" conformation of the molecule. EM studies show the greatest promise here, most likely using nanodisks. We are beginning to understand the structures of the TM helices and their cytosolic extensions, but the biophysics of inside-out signaling in particular requires further study. The role of mechanical force, whether of intracellular (actomyosin motors) or extracellular (shear flow in the vasculature) origin has not been discussed here, but its interplay with the chemical forces that attract cognate molecules is a fascinating field for current and future study [48]. Finally, this chapter has addressed the structural basis of affinity changes within

Table 8.1 Reported structures of integrins, 1996–present

Year	Protein	PDB code	Notes
Wild-type α-I domains			
1996	αM-I, Mg^{2+}	1IDO	
1996	αL-I, Mn^{2+}	1LFA	
1996	αL-I, Mg^{2+}	1ZOO, 1ZOP	
1996	αL-I metal-free	1ZON	
1997	αM-I, Mn^{2+}	1JLM	
1998	αM-I, soaks	1BHO, 1BHQ, 1IDN	Mg^{2+}, Mn^{2+}, free
1998	α2-I	1AOX	
2000	α1-I	1QC5	
2000	α1-I	1CK4	Rat
2000	αL-I	1DGQ	NMR structure
2003	αX-I	1N3Y	
2003	αL-I	1MQ9	High affinity form
I-like domains			
2004	A domain, Factor B	1Q0P	
2010	*Haemophilus* pilus	2WW8	
I domain-ligand complexes			
2000	α2-Collagen	1DZI	
2003	αL-ICAM1	1MQ8	
2005	αL-ICAM3	1T0P	
2008	αL-ICAM5	3BN3	
2013	αM-C3d	4M76	
2013	α1-Collagen	2M32	NMR/HADDOCK model
Engineered I domains/complexes			
2002	αM-I	1MIU, 1MQA	Ile switch
2003	αL-I	1MJN	Intermediate affinity
2003	αM-I	1MF7,1N9Z,1NA5	Modulatory mutants
2009	αL-I	3HI6	Disulfide-bonded intermediate
2011	α1-I	4A0Q	Activating mutation
2011	αL-ICAM-1	3TCX	Mutant high affinity I domain
2013	α2-Collagen	4BJ3	Mutant high affinity I domain
I domain-small molecule/FAB complexes			
2001	αL-LOVASTATIN	1CQP	
2004-2014	αL modulators	1RD4,1XDD,1XDG,1XUO,2ICA, 2O7N 3BQM, 3BQN,3E2M,4IXD,3F74,3F78	
2009/2010	αL-EFALIZUMAB	3EOA,3EOB,3M6F	

(continued)

Table 8.1 (continued)

Year	Protein	PDB code	Notes
2011	αM-FAB	3Q3G,3QA3	
Cytoplasmic Tail-protein complexes			
2003	β3-Talin (chimera)	1MIZ,1MK7,1MK9	
2005	β3-PIP-kinase	1Y19	
2005	αIIb-Filamin	2BP3	
2006	β7-Filamin	2BRQ	
2007	β3-TalinF3	2H7E	NMR
2007	β3-PIP-kinase	2H7D	NMR
2008	β2-Filamin	2JF1	
2008	β3-DOK1	2V76	
2008	β2-P-14-3-3	2V7D	P = Phosphorylation
2009	β1D-Talin2	3V9W	
2010	β3-shc-P	2L1C	
2012	αIIb-CIB1	2LM5	
2012	β1-Acap1	3T9K	
2012	β3-Src	4HXJ	
2013	β4-14-3-3	4HKC	
2013	β1-Acap1	4DX9	
Transmembrane Domains			
2008	β3	2RMZ,2RN0	
2008	αIIb	2K1A	
2009	αIIbβ3	2K9J	
2009	αIIbβ3	2KNC	
2011	β3	2KV9	S–S linked
2012	α2	2L8S	Detergent micelles
2014	αLβ2	2M3E	
2014	β3	2L91	
Cytoplasmic Domains			
2000	αIIb mutant	1DPQ	
2000	αIIb	1DPK	
2002	αIIbβ3	1M8O	
2002	αIIbβ3	1KUP,1KUZ	
2004	αIIbβ3	1S4W,1S4X	Micelles
2008	αL	2K8O	NMR
2011	P-β3	2LJF	Aqueous
2011	αMβ2	2LKE,2LKJ	
2011	P-β3	2LJD,LJE	
2012	αXβ2	2LUV	
α6β4 Intracellular domains/complexes			
1999	β4-FibIII pair	1QG3	
2008	β4-FibIII	2YRZ	NMR

(continued)

Table 8.1 (continued)

Year	Protein	PDB code	Notes
2009	β4-FibIII	3F7Q,3F7R	
2009	β4 Calx	3FQ4,3FSO,3H6A	
2009	β4-Plectin complex	3F7P	
Full-length integrins			
2014	αIIbβ3	4CAK	EM
2009	αVβ3	3IJE	Chimera?
Headpiece/ectodomain			
2001	αVβ3	1JV2	
2002	αVβ3-RGD	1L5G	
2002	αVβ3-Mn^{2+}	1M1X	
2004	αIIbβ3-inhibitor	1TYE	
2008	αIIbβ3 rerefined	2VC2,2VDK,2VDL,2VDM,2VDN,2VDO (supercede 1TY7,1TY3,1TXV,1TY5,1TY6)	
2008	αIIbβ3-Fibrinogen	2VDO,2VDP,2VDQ,2VDR	
2009	αIIbβ3	3FCS	"Complete ectodomain"
2009	αIIbβ3	3FCU	Open conformation
2010	αXβ2	3K6S,3K7L,3K72	
2011	αIIbβ3	3NID,3NIF,3NIG	Antagonist
2012	αVβ3	4DX9	Coiled-coil
2012	αVβ3	4G1M	Rerefined
2012	α5β1-RGD	3VI3,3VI4	
2012	α4β7	3V4P,3V4V	$\alpha\beta$ complex
2013	αIIbβ3-RGD	3ZDX,3ZDY,3ZDZ,3ZE0,3ZE1,3ZE2	
2013	α4β7	4IRZ	
2014	αXβ2	4NEH, 4NEN	

individual integrins. Lateral association (clustering) of integrins in the plasma membrane at sites of ECM contact also plays a major role in integrin signaling, and we still know little about its structural basis and regulation [5].

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