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Previews

Integrin Activation In Vivo and In Silico

A new computational study by Jin et al. (2004, this issue of *Structure*) tests the hypothesis that mechanical force induces the conformational changes leading to the activation of integrins.

Cells are glued to their surroundings through a family of transmembrane receptor proteins known as integrins. The growth, movement, and survival of cells are all dependent on bidirectional signals relayed by integrins across the membrane. Each integrin consists of two noncovalently associated heterogeneous subunits: α and β. In mammalian cells, eighteen α and eight β subunits form 24 different types of integrins, which selectively bind to extracellular matrix proteins such as collagen, fibronectin, and adhesion proteins on the surfaces of other cells. To be capable of binding to their various ligands, integrins must be activated in response to both extracellular and intracellular signals. One such signal is the mechanical force exerted by the cytoskeleton and transmitted through a mechanical linkage that couples the cytoskeleton to integrins.

It is known that integrin activation involves conformational changes (Hynes, 2002). Compelling structural evidence has become available since the ligand bound (active) and unbound (inactive) forms of the integrin α_M I domain were crystallized almost a decade ago by Liddington, Arnaout, and coworkers (Lee et al., 1995). Half of the integrin α subunits have a homologous I domain inserted at the top of the integrin headpiece. The first I domain structures revealed that the 200 amino acids of the protein form a Rossman fold, which consists of a single mostly parallel β-sheet surrounded by seven α-helices. The major ligand binding site is located at the top of the domain and termed the metal-ion-dependent adhesion site (MIDAS), for it acquires a divalent metal ion recognized by the ligands. Compared to the unliganded form, the liganded α_M I domain exhibits a few conformational changes that were attributed to the ligand binding; most notably, the C-terminal α-helix shifts 10 Å towards the tailpiece. The movement is linked to the rearrangement of loops bearing the MIDAS residues. Similar conformational changes have been observed for integrin α2 I domain structures subsequently (Emsley et al., 2000), confirming the notion that the C-terminal helix shift is a key feature of the activated integrin I domains. By introducing disulfide bridges that prevent the movement of the C-terminal helix, Springer and colleagues (Jin et al., 2004) have successfully locked integrin αL in states with high affinity (active), low affinity (inactive), or intermediate affinity (Lu et al., 2001; Shimaoka et al., 2003).

The observed conformational changes of α I domains led to the hypothesis that the activation of integrin can be regulated by stretching the C-terminal helix. Steered molecular dynamics (SMD) is a method capable of testing the hypothesis. The approach applies an external force to a simulated protein and monitors the corresponding mechanical response (Isralewitz et al., 2001). A typical application of SMD is one recently published (Gao et al., 2003), which tested the stability of the extracellular matrix adhesion protein fibronectin against unraveling. In this issue of *Structure*, Jin et al. (2004) report SMD simulations that examine the role of the mechanical force in regulating the activation of the integrin α I domains through stretching induced conformational changes. The authors attached a spring to the C-terminal α-helix of four individual α I domains (αM, αL, α1, and α2) in inactive forms, and stretched the α-helices along the helical axis.

Consistent with observed crystal structures, the resulting trajectories were grouped into three physiologically relevant forms: inactive (closed), intermediate, and active (open) (Figure 1). The three forms can be distinguished by a hydrophobic ratchet pocket facing the C-terminal α-helix, where in the inactive form a conserved Leu residue on the C-terminus (see Figure 1) is buried in the pocket.

As the helix of αL or the αM I domain is pulled by about 6 Å, a Phe on the C-terminus replaces the Leu, stabilizing an intermediate form previously observed only in the structure of a mutated αL domain. Interestingly, half of integrin I domains, e.g., α1 and α2, possess a charged residue Glu instead of the Phe at the same position and do not exhibit the intermediate.

Stretching and further shifting the helix by about 5 Å leads to the active form. In this form, yet another hydrophobic residue has swung into the ratchet pocket replacing Phe, the previous occupant. The simulated movement of the helix induces changes in loops bearing the MIDAS coordinating residues similar to what had been observed in the activated form crystal structures. Overall, the new SMD study strongly supports the hypothesis that integrin α I domains can be activated by pulling the C-terminal helix.

This is the second study that employed SMD to investigate integrins; a recent prior study investigated the adhesion of integrins to fibronectin involving the Arg-Gly-Asp (RGD) loop of the latter. Integrin β subunits contain a homologous I domain known as the β I-like domain at the top of the β subunit. In case of integrins without an α I domain such as αVβ3, the I-like domain participates in RGD loop binding at the MIDAS site (Xiong et al., 2002). In addition to the MIDAS site, however, the I-like domain contains two adjacent metal ion binding sites. Molecular dynamics simulations of the αVβ3/RGD-ligand complex revealed that the Asp residue of the RGD loop contacts one of the adjacent metal ions in addition to contacting the MIDAS ion (Craig et al., 2004). Using SMD, Craig et al. (2004) further demonstrated a key aspect of the integrin-ligand complex. A tight coordination of the MIDAS ion and RGD loop Asp residue blocks the access of free water molecules to the contact, thereby stabilizing the interaction. In con-

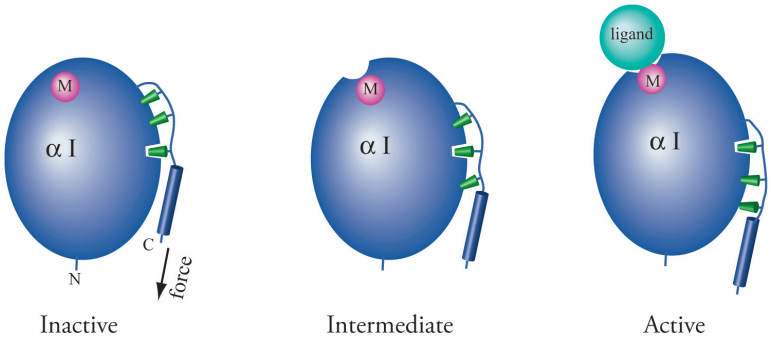

Figure 1. Conformational States of the Integrin α_L I Domain Regulated by Mechanical Force

The C-terminal α-helix, represented as a blue cylinder, shifts downwards by about 10 Å upon application of the activating force. The movement triggers allosteric changes around the major binding site, where a ligand binds to the protein through a divalent metal ion (colored in purple). Three noncontiguous hydrophobic amino acids, represented as green cones, successively occupy a hydrophobic cavity that serves as a mechanical ratchet pocket for stabilizing the inactive, intermediate, and active forms. See the article by Jin et. al. (2004) in this issue.

trast, salt bridges formed between RGD ligand and αV headpiece are constantly under attack from surrounding water molecules and are disrupted relatively easily upon stress.

Two 18 nm long legs connect the α I and/or β I-like domains of integrins to their cytoplasmic domains, where the integrins associate with cytoskeleton coupled mechanical linkages. Stressing either end of integrins, namely the I/I-like domains or the cytoplasmic domains, creates allosteric changes sensed by the cell and the extracellular matrix as signals. But how do these conformational changes propagate within the signaling machines? Only a combination of crystallography and computational modeling, in particular SMD, can ultimately answer the question.

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Selected Reading

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