MECHANISMS CONTROLLING RECEPTOR ACTIVITY OF INTEGRINS AND THEIR INTERACTIONS WITH PROTEIN LIGANDS

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Cells, in order to sense and respond to its environment and for the environment to influence cellular functions (including cell growth and movement), require bidirectional signaling across the plasma membrane that has to be mediated by receptors and other structures. It became widely appreciated that many of the cell surface receptors that mediate cell-cell and cell-extracellular matrix (ECM) interactions were structurally and functionally related. That is why they are called "integrins" to reflect the capacity of members of this family to integrate the extracellular and intracellular environment. Integrin-mediated interactions mediate inside-out (intracellular to extracellular) and outside-in (extracellular to intracellular) signaling.

The β_3 subfamily of integrin heterodimeric adhesion receptors consists of two highly homologous members, αIIbβ3 and αVβ3. αIIbβ3 is essential for platelet aggregation and, thereby, controls platelet function in thrombosis and hemostasis. αVβ3 is expressed on many cells, where it influences cell migration and impacts on angiogenesis, restenosis, tumor cell invasion, and atherosclerosis. These two integrins share the same β subunit, β3, but have distinct α subunits; αIIb and αV exhibit about 36% primary amino acid sequence identity. Their common macromolecular ligands such as fibrinogen, fibronectin, thrombospondin, von Willebrand factor, and vitronectin contain Arg-Gly-Asp (RGD) sequences, and RGD-containing peptides inhibit binding of these ligands to both receptors.

To develop an understanding of the molecular basis of functional differences between integrins αVβ3 and αIIbβ3, we produced and characterized soluble recombinant "mini-receptors" and evaluated their ligand binding activity. They corresponded to the "head" of both receptors consisting of the relevant β-propeller domain and the β3A domain. After refolding, recombinant αV(1–438), αIIb(1–438), and β3(109–352) adopted their native conformation as evaluated by binding monoclonal antibodies and fibrinogen. Our results demonstrate that: (a) the ligand binding specificity can be fully reconstructed by association of αV or αIIb β-propeller and β3 A domain. When examined for their ligand-binding properties and for their expression of anti-integrin antibody epitopes, each of these recombinant mini-receptors, namely αV(1–438)/ β3(109–352) and αIIb(1–438)/β3(109–352), displayed the antibody (LM-609, PAC-1) binding characteristics of their corresponding receptors. The binding constant (K_D) of the αV and αIIb β-propellers for the β3A domain fragment was 3.63 and 3.95 × 10^{-7} M, respectively. (b) Both mini-receptors reacted with fibrinogen showing significantly increased binding affinity compared to that of the β3A domain alone. The αV(1–438)/β3(109–352) complex had the same affinity while the αIIb(1–438)/β3(109–352) had approximately a 10-fold lower apparent binding affinity for fibrinogen than the native receptors. (c) Association of β-propellers with β3A domain was influenced by Ca^{+2}. Since mutation of the metal binding sites in the β3 A domain did not hamper their interaction with the β-propeller domains, these sites do not contribute the cations required for optimal formation of the α/β interface.