RECONSTRUCTION OF THE LIGAND BINDING DOMAIN OF CYTOADHESINS αVβ3 AND αIIbβ3

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To develop an understanding of molecular basis of functional differences between αVβ3 and αIIbβ3, in the present work we produced soluble, recombinant "minireceptors" – β3(109–352)/αV(1–438) and β3(109 –352)/αIIb(1–438) and evaluated their functions. They corresponded to the main area of ligand binding that lies between the β-propeller of the α subunit and the A-domain in the β3 subunit. After refolding, recombinant "minireceptors" adopted their native conformation as evaluated by circular dichroism spectroscopy, MALDI-TOF mass spectrometry and binding several monoclonal antibodies. 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. Each of these recombinant minireceptors, namely αV(1–438)/β3(109–352) and αIIb(1–438)/ β3(109–352) are recognized by monoclonal antibodies LM-609 and PAC-1, respectively.

(b) Both minireceptors reacted with fibrinogen showing significantly increased binding affinity comparing to that of the β3 A domain alone. The αV(1–438)/β3(109–352) complex had the same while the αIIb(1–438)/β3(109–352) approximately 10-fold lower apparent binding affinity as the native receptors. Mutations of the β3 A-domain in MIDAS and ADMIDAS in both minireceptors significantly reduced their binding affinity for fibrinogen. The effect of mutation within the LIMBS on fibrinogen binding was much less pronounced.

(c) Association of β-propeller domain with β3 A domain in both cases is critically depended upon the presence of Ca^{+2}. Since mutation of the metal binding sites in the β3 A domain did not hamper their interaction with β-propeller domains, these sites do not seem to be involved in formation of the α/β interface. Since four Ca^{2+} binding sites of β-propeller domain are located at the propeller's bottom, opposite to the α/β interface, they are also not expected to participate directly but rather indirectly in the interaction of both domains.