

P021 Role of the ADMIDAS Cation, Binding Site in Ligand Binding by Integrin $\alpha 5\beta 1$

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Integrin-ligand interactions are regulated in a complex manner by divalent cations: metal ions influence the activation state of the integrin and also participate directly in ligand recognition. Multiple cation-binding sites are found in both α and β subunits. A key cation-binding site that lies in the β subunit A domain is known as the metal-ion dependent adhesion site (MIDAS). Recent X-ray crystal structures of integrin $\alpha V\beta 3$ have identified a novel cation binding site in this domain, known as the ADMIDAS (adjacent to MIDAS). The role of this novel site in ligand recognition has yet to be elucidated.

Using the interaction between $\alpha 5\beta 1$ and fibronectin as a model system, we show that: (i) Mutation of residues that form the ADMIDAS site inhibits ligand binding but this effect can be partially rescued by the use of activating monoclonal antibodies. (ii) The ADMIDAS mutants have decreased expression of activation epitopes recognized by 12G10, 15/7 and HUTS-4, suggesting that the ADMIDAS is important for stabilising the active conformation of the integrin. Consistent with this suggestion, the ADMIDAS mutations markedly increased the dissociation rate of the integrin/fibronectin complex. (iii) Mutation of the ADMIDAS residues reduces the allosteric inhibition of Mn^{2+} -supported ligand binding by Ca^{2+} , suggesting that the ADMIDAS is a Ca^{2+} -binding site involved in the inhibition of Mn^{2+} -supported ligand binding. (iv) Mutations of the ADMIDAS site perturb transduction of a conformational change from the MIDAS through the C-terminal helix region of the βA domain to the underlying hybrid domain, implying an important role for this site in receptor signalling.