

P022 Influence of di-phenylalanine residues within α -integrin GFFKR motif on integrin $\alpha_{IIb}\beta_3$ activation state: a parallel approach in platelets and CHO cells.

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The membrane proximal domain KxGFFKR of α -integrin subunits is highly conserved, suggesting a pivotal role for this region in integrin function. To ascertain the contribution of the two phenylalanine residues in the modulation of integrin affinity state, a palmitoylated peptide corresponding to the platelet specific integrin $\alpha_{IIb}\beta_3$ KVGFFKR sequence was synthesised (Pal-FF). Also, peptides with a single (Pal-AF) and a double substitution (Pal-AA) were studied. In parallel, single (AF) or double (AA) alanine substitutions were introduced to α -subunits and co-expressed with β_3 in CHO cells. In platelet studies, both Pal-FF and Pal-AF dose-dependently induced platelet aggregation and thromboxane synthesis, while Pal-AA itself induced minimal aggregation. Furthermore, Pal-AA inhibited thrombin-induced aggregation, thromboxane synthesis, Pac-1 binding and α -granule secretion. In the stable cell system, Pac-1 binding to the FF and AF cells was not observed indicating $\alpha_{IIb}\beta_3$ is in a resting conformation, while Pac-1 bound to the AA cells confirming $\alpha_{IIb}\beta_3$ is in an activated conformation. The AA cells displayed increased spreading and adhesion to immobilised fibrinogen when compared to the FF and AF cells. These studies emphasise the di-phenylalanine influence on the activation state of the integrin.