

P003 Inhibition of kinase dimerization by a structure-derived peptide
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Receptor tyrosines kinases (RTK) are activated by autophosphorylation. A prerequisite for autophosphorylation is dimerization of the kinase domains. Targeting the unique dimerization process of a kinase, should lead to specific inhibition in contrast to ATP mimics which are commonly applied for this purpose. As a representative target for such inhibition we found the conserved α -helix D in the kinase structure. The position of the α D-helix as part of the hinge region determines the inactive or active state of the kinase. The peptide comprising 15 residues of the α D-helix of the insulin receptor inhibits the kinases of IR and IGF-1R in autophosphorylation reactions. In addition the phosphoryl transfer in substrate phosphorylation reactions is efficiently inhibited in the 10 μ M range. The inhibitor did not decrease the affinity of IR/IGF-1R kinases for neither ATP nor substrates significantly. Rather, at 24 μ M peptide concentration the k_{cat} of substrate phosphorylations catalyzed by the IR kinase was reduced from $67.4 \pm 3.4 \text{ min}^{-1}$ to $30.28 \pm 1.5 \text{ min}^{-1}$. The specificity of the inhibitor towards the kinases of the IR family has been proven with a randomized peptide and by the use of a unrelated kinase (Akt). In both cases no effect has been observed. Remarkably, once activated kinase is no longer inhibited by the peptide in standard substrate phosphorylations with polyE₄Y. However, by choosing an inactive kinase mutant (IRKD-D/A) as a substrate inhibition was restored. IRKD-D/A serves primarily as a dimerization partner. Thus we conclude that the peptide disrupts the kinase dimerization process.