INVESTIGATION OF INSULIN-LIKE RECEPTOR SYSTEMS

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SUMMARY

The insulin and insulin-like growth factor receptor (IR and IGF-1R respectively) networks are ancient and fundamental systems that control growth and metabolism in multicellular organisms. This thesis has examined several aspects of this field focusing on mammalian receptor biology and a comparison of the similarities and differences between the insulin and IGF receptor signalling systems.

The insulin receptor family of proteins consist of eleven structural domains, of which the extracellular domains contain all the ligand binding and specificity determinants. The insert domain, within the extracellular region is the least understood of all the domains, and it has no similarity to any other protein sequence. It does however contain the cleavage site which separates the receptor into two subunits and also a small stretch of residues shown to directly contact bound ligand and which is absolutely required for ligand binding in short recombinant forms of the receptor. In addition, the human insulin receptor, expressed as one of two isoforms, A and B, results in the exclusion or inclusion of 12 amino acids directly adjacent to the ligand contacting amino acids in the insert domain. The A isoform lacking exon11 is expressed ubiquitously and the B isoform containing exon11 is co-expressed mainly in the traditional insulin responsive tissues of liver, muscle, adipocytes and kidney, where it is the dominant isoform.

In this thesis recombinant insert domain was expressed in a bacterial system in an attempt to purify folded protein suitable for NMR structural analysis. The results of the expression studies indicated that the insert domain was unstructured in isolation and was unable to be adequately refolded by all conditions tried, although hydrophobic conditions appeared to partially stabilize the structure. The overall conclusions of this project were that the Insert domain is likely to have limited structure, and probably buried within the receptor, and therefore requires the presence of the rest of the extracellular domains to adopt its correct structure.

A comparison of the ligand binding and phsophorylation potential between the two human isoforms of the insulin receptor was made. A competition binding assay using europium labelled insulin was developed, that found that both IGF-1 and IGF-2 had an increased affinity for the hIR-A, but insulin had a slightly reduced affinity. These results differ from the established literature in the raw values, however the relative ratios of binding strength are consistent. The

most likely reason for this is that the europium labelled insulin has a different mode of binding the receptors due to the location of the europium chelate. Interestingly, using europium labelled IGF-1 produced results nearly identical to those of conventional competition assays.

Phosphorylation assays indicated that the hIR-B isoform was more responsive than hIR-A. Even though IGF-2 and IGF-1 had improved affinity for hIR-A, the level of phosphorylation was not as high. The ability of each growth factor to promote cellular proliferation correlated well with the relative strength of binding and activation of the receptor.

The regions of the IR and IGF-1R involved in binding substrates and regulators are predominantly found in the juxtamembrane domain and the C-terminal domain, which contain several potential tyrosine and serine phosphorylation target sequences. In this study the effect of mutations in unique tyrosine residues and other residues in the C-terminal domain of the hIGF-1R was investigated. Results of time-course phosphorylation assays showed that mutation of Tyrosine^{1251} to phenylalanine caused hyperphosphorylation of the receptor and increased proliferation, which was caused by deregulation of a tyrosine phosphatase. A Tyrosine^{1250} to phenylalanine mutation had altered kinetics of phosphorylation, displaying an unchanging rate of phosphorylation over time after ligand stimulation. However, proliferation was unaltered, indicating that even under extended exposure to ligand, the initial strength of receptor activation is more critical to affecting the biological response.

The *Caenorhabditis elegans* insulin-like peptide family is a very large family consisting of possibly 38 peptides likely to be both agonists and antagonists of Daf-2 Receptor (IR homologue) signalling. Comparative modelling of all 38 peptides was performed based on the known structures of mammalian peptides. The overall results indicated that good quality models of ins peptides could be made despite the low sequence similarity with the templates. This suggested that it is the conformational shape of the molecule allowable by the individual residues that is most important when modelling and not having a perfect sequence match.