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UNIVERSITY OF ADELAIDE

# **INVESTIGATION OF INSULIN-LIKE RECEPTOR SYSTEMS**



**THE UNIVERSITY  
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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 phosphorylation 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<sup>1251</sup> to phenylalanine caused hyperphosphorylation of the receptor and increased proliferation, which was caused by deregulation of a tyrosine phosphatase. A Tyrosine<sup>1250</sup> 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 insulin 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.

## CHAPTER 1 INTRODUCTION

1.1 COMPONENTS OF THE INSULIN/IGF SYSTEM	4
1.2 STRUCTURAL BIOLOGY OF INSULIN/IGF RECEPTORS	5
1.2.1 Receptor Gene Structure	5
1.2.2 Domain Organization	6
1.2.3 Receptor Biosynthesis	6
1.2.3.1 Hybrid Receptors	7
1.2.4 Three-dimensional Structure of the Receptor	8
1.2.4.1 Anti-receptor Antibodies	9
1.2.5 Molecular basis of ligand binding	10
1.2.5.1 Role of the L1 Domain	10
1.2.5.2 Role of the Cys-Rich Domain	11
1.2.5.3 Role of the L2 Domain	11
1.2.5.4 Role of the Fibronectin Type-III Domains	12
1.2.5.5 Role of the Insert Domain	12
1.2.5.6 Different Mechanisms of Binding between the Insulin Receptor and IGF-IR	13
1.2.5.7 Minimized Ligand Binding Receptor	14
1.2.6 Structure and Function of Insulin-like Proteins	15
1.2.7 Insulin-like Growth Factor II Receptor	16
1.2.8 Insulin-like Growth Factor Binding Proteins	16
1.3 RECEPTOR SIGNAL TRANSDUCTION: SIGNALLING AND SPECIFICITY	17
1.3.1 Tyrosine Kinase Activation	17
1.3.2 Phosphorylation and Activation of Common Receptor Substrates	17
1.3.2.1 Activation of Pathways Controlling Biological Effects	18
1.3.2.1.1 Phosphatidylinositol 3-kinase Pathway	18
1.3.2.1.2 Mitogen Activated Kinase Pathway	20
1.3.2.2 Separating the IR and IGF-IR Specific Pathways and Substrates	20
1.4 ROLE OF INSULIN-LIKE PROTEINS IN DISEASE	22
1.4.1 Diabetes	22
1.4.1.1 Normal Regulation of Glucose Metabolism	22
1.4.1.2 Molecular Basis of Insulin Dependent Diabetes Mellitus	22
1.4.1.3 Molecular Basis of Non-Insulin Dependent Diabetes Mellitus	23
1.4.1.3.1 Is there a Role for Altered Expression of the IR Isoforms in NIDDM?	24
1.4.2 Cancer	25
1.4.2.1 Evidence of a Role for the IGF-IR in Transformation and Maintenance of Tumours	25
1.4.2.2 Breast Cancer	26
1.4.2.2.1 Is there a Role for Altered Expression of IR Isoforms in Cancer?	26
1.4.3 Targeting the Insulin/IGF system	27
1.5 SUMMARY AND PROJECT AIMS	29

## CHAPTER 2 MATERIALS AND METHODS

2.1 ABBREVIATIONS	31
2.2 MATERIALS	32
2.2.1 General Materials	32
2.2.2 Chemicals and Reagents	32
2.2.3 Enzymes	34
2.2.4 Antibodies	34
2.2.5 Bacterial Strains	34
2.2.6 Tissue Culture Cell Lines	35
2.2.7 Bacterial Cloning and Protein Expression Vectors	35
2.2.8 Mammalian Cell Culture Expression and Reporter Vectors	35
2.2.9 PCR Primers	35
2.2.10 Commercial Kits	36
2.2.11 Molecular Weight Standards	36
2.2.11.1 DNA Markers	36
2.2.11.2 Protein Markers	37
2.2.12 Solutions	37
2.2.13 Online and Computing Resources	38
2.3 METHODS	40
2.3.1 Bacterial Methods	40
2.3.1.1 Making Glycerol Stocks	40
2.3.1.2 Calcium Chloride Competent Cells	40
2.3.1.3 Transformation of Competent Cells by Heat Shock	40
2.3.1.4 Large Scale Midiprep Kit	40
2.3.1.5 Medium Scale Plasmid Preparation	41
2.3.1.6 Small scale kit	41
2.3.1.7 Small Scale Plasmid Preparation	42

2.3.2 Molecular Methods	42
2.3.2.1 Agarose Gel Electrophoresis	42
2.3.2.2 Purification of Linear DNA fragments	42
2.3.2.3 Restriction Endonuclease Digestion of Plasmid DNA	42
2.3.2.4 Ligation Reactions	43
2.3.2.5 RNA purification from cells in tissue culture	43
2.3.2.6 mRNA Purification from whole <i>C. elegans</i> RNA	43
2.3.2.7 Reverse Transcription	44
2.3.2.8 Primer Design	44
2.3.2.9 Polymerase Chain Reaction (PCR)	44
2.3.2.10 Cycle Sequencing of Plasmid DNA	45
2.3.3 In vitro Methods	45
2.3.3.1 Culture of Mammalian Cells	45
2.3.3.2 Cell counting using cytometer	46
2.3.3.3 Transfection of cells using Lipofectamine +™	46
2.3.3.4 Methylene Blue cell viability assay	46
2.3.3.5 Cell titre Glo assay	47
2.3.3.6 Cell Lysis	47
2.3.3.7 Basic FACS analysis	47
2.3.3.8 Phosphorylation Assay	48
2.3.3.9 Time-course Phosphorylation Assay	48
2.3.4 Protein Methods	49
2.3.4.1 Western Blot	49
2.3.4.2 Europium-labelling proteins	49
2.3.4.3 Preparation of 96-well Plates with anti-receptor antibody	50
2.3.4.4 Insulin Receptor ELISA	50
2.3.4.5 Competition Assay with eu-labeled growth factors	50
2.3.4.6 <sup>125</sup> I growth factor competition assay	51
2.3.4.7 Detection of Tyrosine Phosphorylation using eu-labeled anti-phosphotyrosine Antibody	51
2.3.4.8 Quantitation of Protein concentration	52
2.3.4.9 SDS-PAGE Electrophoresis	52
2.3.4.10 Ni-Affinity Chromatography	52
2.3.4.11 High Performance Liquid Chromatography Analysis	53
2.3.4.12 Thrombin Cleavage of fusion proteins	53
2.3.4.13 Proteinase K Digestion	53
2.3.4.14 NMR Spectroscopy	54
2.3.5 Computational Methods for Comparative Modelling	54
2.3.5.1 Protein Sequence Alignments	54
2.3.5.2 Building the Comparative Models	54
2.3.5.3 Simulated Annealing	54
2.3.5.4 Model Evaluation	55
<b>CHAPTER 3 EXPRESSION OF INSERT DOMAIN PROTEINS</b>	<b>56</b>
3.1 INTRODUCTION	56
3.1.1 Project Summary and Aims	58
3.2 RESULTS	59
3.2.1 Construction of recombinant insert domain expressing vectors	59
3.2.2 PROTEIN EXPRESSION	60
3.2.2.1 Expression of Thioredoxin-Insert Domain fusion protein	60
3.2.2.2 Expression of Thioredoxin-Fibronectin 3-Insert Domain fusion protein	61
3.2.3 PROTEIN PURIFICATION	62
3.2.3.1 TRX-ID Purification by Ni-affinity chromatography	62
3.2.3.2 TRX-ID Purification by Resource Q chromatography	63
3.2.3.3 Thrombin Kinase digestion	63
3.2.3.4 HPLC Purification of ID	64
3.2.3.5 NMR of Purified ID	64
3.2.4 REFOLDING STUDIES	65
3.2.4.1 Analysis of the State of ID by Gel Filtration Chromatography	65
3.2.4.2 Proteinase K Assay	65
3.2.4.3 Alteration of Refolding Conditions	65
3.3 DISCUSSION	67
<b>CHAPTER 4 CROSS-REACTIVITY OF GROWTH FACTORS IN THE INSULIN SYSTEM</b>	<b>70</b>
4.1 INTRODUCTION	70
4.1.1 Project Summary	71
4.2 RESULTS AND DISCUSSION	72
4.2.1 Construction of Full Length hIR Isoforms in Mammalian Expression Vectors	72
4.2.2 Construction of Recombinant hIR-B Ectodomain with a C-terminal Leucine Zipper	72
4.2.3 Creation of Cell Lines Expressing Full Length hIR Isoforms	73

4.2.4	Creation of a Cell Line Expressing Recombinant hIR-B EDZIP Isoform	74
4.2.5	Development of a Receptor Competition Assay using Europium Labeled Ligands	75
4.2.5.1	Europium Labelling Insulin, IGF-1 and IGF-2	75
4.2.5.2	Optimization of the Europium Competition Assay	76
4.2.6	Comparison of Ligand Binding between Full-length hIR Isoforms in the Europium Competition Assay	77
4.2.6.1	The C and D domains of IGF-1 and IGF-2 Determine Receptor Specificity	79
4.2.7	Comparison of Ligand Binding between Recombinant Soluble Ectodomain Isoforms in the Europium Competition Assay.	80
4.2.8	Comparison of Ligand Binding between Recombinant Soluble Ectodomain ZIP Isoforms in the Europium Competition Assay.	80
4.2.9	Phosphorylation Assays	81
4.2.10	Proliferation Assays	83
4.2.11	Anti-Apoptosis Assays	84
4.2.12	Creation of antibody that can differentiate between the insulin receptor isoforms	85
4.2.12.1	Characterisation of the polyclonal anti-exon 1 serum	85
4.3	SUMMARY AND CONCLUSION	87
<b>CHAPTER 5 C-TERMINAL MUTANTS OF THE IGF-1R</b>		<b>89</b>
5.1	INTRODUCTION	89
5.1.1	Project Summary and Aims	90
5.2	RESULTS AND DISCUSSION	91
5.2.1	Construction of hIGF-1R Cytoplasmic Mutant Plasmids	91
5.2.2	Creation of Stable Cell Lines Expressing Mutants	91
5.2.3	Competition Assays on Solubilised Receptors with IGF-1, 2	94
5.2.4	Mutant Receptor Complexes Show Altered Amounts of Tyrosine Phosphorylation in Response to IGF-1.	95
5.2.5	Effect of Phosphatase Inhibition on Tyrosine Phosphorylation	98
5.2.6	Effect of Mutations on the Proliferative Potential of the Receptor	99
5.2.7	PTP-ID Localization is not Affected in the Y1251F or Y1250F Mutants	101
5.3	SUMMARY AND CONCLUSION	102
<b>CHAPTER 6 COMPARATIVE MODELLING OF C. ELEGANS INSULIN-LIKE PEPTIDES</b>		<b>103</b>
6.1	INTRODUCTION	103
6.1.1	Project Summary and Aims	105
6.2	RESULTS AND DISCUSSION	106
6.2.1	Analysis of Ins Peptide Sequences	106
6.2.2	Comparative Modelling of Ins Peptides	106
6.2.2.1	Selection of Modelling Templates	107
6.2.2.2	Target-Template Alignment	108
6.2.2.3	Building the Models	109
6.2.2.4	Refinement of the Models	111
6.2.2.5	Evaluation of the Models	111
6.2.2.6	Model Comparisons	112
6.2.3	RT-PCR of Ins Peptides	114
6.2.4	Cloning and Sequencing of Ins-6, 11, 17	116
6.3	SUMMARY AND CONCLUSION	118
<b>CHAPTER 7 FINAL DISCUSSION</b>		<b>119</b>
7.1	ROLE OF THE INSERT DOMAIN IN RECEPTOR FUNCTION	119
7.2	CROSS-REACTIVITY OF IGF-2 WITH THE INSULIN RECEPTOR	121
7.3	ROLE OF TYROSINE 1250 AND 1251 IN IGF-1R PHOSPHORYLATION	122
7.4	THE RELATIONSHIP OF INSULIN-LIKE PEPTIDES IN C. ELEGANS WITH MAMMALIAN INSULIN-LIKE PROTEINS	123
<b>CHAPTER 8 REFERENCES</b>		<b>125</b>
<b>APPENDIX</b>		<b>139</b>