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The insulin-like growth factor mutation database (*IGFmdb*)

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ABSTRACT

Insulin-like growth factors (IGF-I and IGF-II), and insulin are evolutionarily conserved hormonal regulators of eukaryotic growth and development. Through interactions with their cognate receptors, all three molecules can influence cellular growth, proliferation, differentiation, migration, and survival, as well as metabolic processes. As such, perturbations in signaling by IGFs and insulin are a well-documented cause of altered growth, development and survival during both embryonic and post-natal life.

A key approach in understanding how IGFs and insulin elicit their biological effects has been through identifying structural features of the ligands that influence their receptor interactions. Over the years, the study of many hundreds of specifically engineered IGF and insulin analogues has provided a wealth of knowledge about how specific residues of these ligands contribute to ligand:receptor interactions. Some analogues have even provided the basis for designing therapeutic agents for the treatment of IGF and insulin-related diseases.

As the list of IGF and insulin analogues continues to grow we find that, while many have been produced and studied, it would be of considerable value to have a central repository from which information about specific analogues and their receptor binding data were readily available in an easily searchable and comparable format. To address this, we have created the "Insulin-like growth factor mutation database" *(IGFmdb)*. The *IGFmdb* is a web-based curated database of annotated ligand analogues and their receptor binding affinities that can be accessed via http://www.adelaide.edu.au/igfmutation.

Currently the *IGFmdb* contains receptor-binding data for 67 IGF-II analogues that were publicly accessible prior to 2012, as well as 67 IGF-I analogues, including all of those produced and characterised in our laboratory. A small number of these are IGF species homologues. There are also 32 insulin analogues within *IGFmdb* that were reported within the included IGF analogue studies, representing only a small fraction of existing insulin mutants. Future developments of the *IGFmdb* will incorporate receptor-binding data for all publicly accessible IGF-I analogues and the data will be expanded to include IGF-binding protein (IGFBP) binding affinities.

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1. Introduction

The insulin-like growth factor (IGF) system is a complex biological signalling network that promotes important biological processes including cell growth, proliferation, differentiation, migration and survival against apoptosis. These activities are important for tissue development, bone growth, brain development, memory function and energy metabolism, which are linked to organism size and longevity. Dysregulation of insulin signalling leads to diabetes, whereas perturbation of the IGF system leads to diseases such as acromegaly, arising from high circulating IGF levels, or Laron dwarfism, due to low circulating IGF levels [1,2]. Increased IGF signalling potentiates cancer cell growth, survival against chemotherapy and metastasis [3]. Not surprisingly, an enormous effort has been made to understand the mechanisms underlying IGF and insulin action with the aim to develop therapies for these diseases. This has included the use of IGF and insulin analogues to investigate the molecular determinants of receptor interactions.

1.1. Ligands of the IGF system

The IGF system includes three evolutionarily conserved, structurally similar polypeptide ligands: IGF-I, IGF-II and insulin [4]. IGF-I and IGF-II are homologous single chain polypeptides, containing 70 and 67 amino acids, respectively [5]. Both are composed of four distinct structural domains identified as the B, C, A and D domains (Fig. 1). IGF-I and IGF-II also share a high degree of structural similarity to insulin. However, insulin in its processed form is comprised of two independent, disulfide-bonded peptide chains (termed the B and A chains) of 30 and 21 residues respectively. Spatially and structurally, the B and A domains of the IGFs are arranged similarly to the B and A chains of insulin and contain three alpha helices held together by two inter-domain disulphide bonds and a single intra-domain disulphide bond. The C and D domains of IGFs, which are not found in mature insulin, are relatively unstructured [4,5] (Fig. 1).

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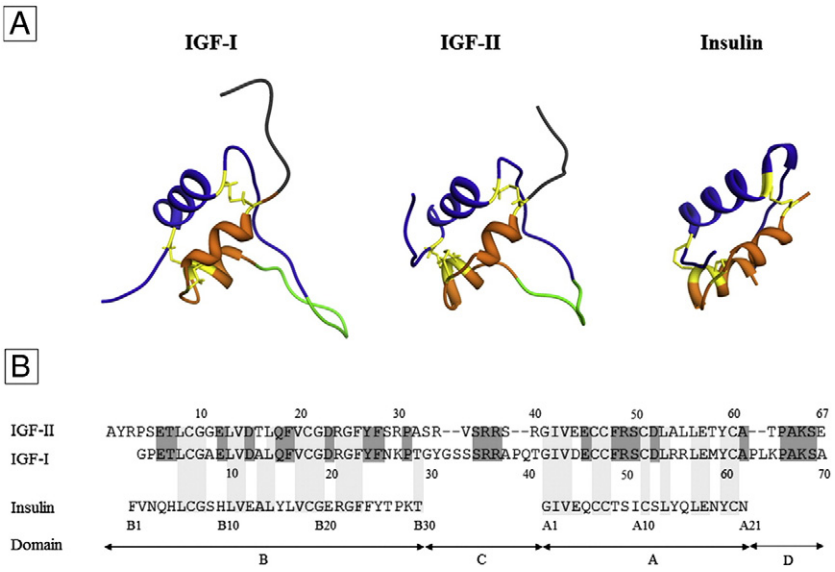

Fig. 1. The ribbon structures of IGF-I, IGF-II and insulin (A) and the sequence alignment of IGF-I, IGF-II and insulin (B): in (A) the domains B, C, A and D are highlighted in blue, green, orange and grey respectively. Cysteine residues involved in the formation of disulphide bonds are presented in yellow. These disulphide bonds are located between C6–C48, C18–C61 and C47–C52 in IGF-I [36], C9–C47, C21–C60 and C46–C51 of IGF-II [37] and C^{B7}–C^{A7}, C^{B19}–C^{A20}, and C^{A6}–C^{A11} of insulin [16]). Images of IGF-I, IGF-II and insulin were generated using the following PDB coordinate files; 1PMX.pdb for IGF-I [36], 1IGL.pdb for IGF-II [37] and 1HIU.pdb for insulin [51]. In the sequence alignment (B), the residues conserved between IGFs and insulin are highlighted in light grey while residues conserved between IGF-I and IGF-II are highlighted in dark grey. The different domains are indicated below. The sequence alignment is adapted from [27].

The bioavailability of IGF-I and IGF-II but not insulin is regulated by a family of six high affinity binding proteins (IGFBP) [6]. Within the circulation, IGFs are held in a ternary complex with an IGFBP (IGFBP-3 or -5) and the acid labile subunit (ALS). This 150 kDa complex prolongs the half-life of IGFs from minutes to hours [7].

1.2. Receptors of the IGF system

The IGF system consists of three transmembrane receptors. IGF-I and IGF-II bind with high affinity to the type 1 IGF receptor (IGF-1R) [8] and promote mitogenic signalling. Despite its similarity to IGF structure, insulin has ~1000 fold lower affinity for the IGF-1R. However, it binds the insulin receptor (IR) with high affinity to promote predominantly metabolic signalling [4]. IGF-II also binds to the type 2 IGF receptor (IGF-2R), a single chain 15 domain transmembrane receptor, which regulates IGF-II levels by targeting IGF-II for lysosomal degradation [9–11].

The IR occurs as one of two splice isoforms (IR-A and IR-B) that differ by the presence (IR-B) or absence (IR-A) of 12 amino acids (encoded by exon 11) located at the very C-terminal end of the alpha subunit [12,13]. Both A- and B- isoforms bind insulin with high affinities and predominantly give rise to metabolic signalling outcomes. Interestingly, IGF-I has low affinity for both IR isoforms, whereas IGF-II has low affinity for the IR-B but binds with high affinity to the IR-A (only a 2.8- to 6.5-fold lower affinity than insulin) [13,14]. IGF-II promotes mitogenic signalling upon binding the IR-A. Both IGF-II and IR-A are expressed in foetal tissues and their expression is also upregulated in cancer, suggesting a role for IGF-II/IR-A signalling in development and cancer. A final layer of complexity arises in cells expressing both IGF-1R and IR receptors as the monomers of each can dimerise, to form IGF-1R/IR hybrid receptors. Hybrid receptors are similar to the IGF-1R in that they only have a high affinity for IGF-I and IGF-II [15]. The physiological role of IGF-1R/IR hybrid receptors is yet to be elucidated.

The IGF-1R and IR are members of the receptor tyrosine kinase superfamily [16]. Structurally, both receptors employ a covalent, dimeric ($\alpha 2\beta 2$) arrangement in which $\alpha\beta$-monomers dimerise and form disulphide bonds to give the final, $\alpha 2\beta 2$ arrangement [8]. For both receptors, the α-subunit is entirely extracellular and provides ligand binding sites, while the β-subunit spans the cell membrane with a small, amino-terminal portion on the extracellular side and the remainder (including the tyrosine kinase domain) on the intracellular side. The crystal structure of the IR ectodomain reveals a folded over structure with two potential binding pockets [17,18]. Unfortunately, to date there is no structure of an IGF:IGF-1R or insulin:IR complex. Our current understanding of the ligand:receptor interaction is derived from biophysical and receptor binding analyses and mathematical and molecular modelling [19–21], which demonstrate that the stoichiometry of binding is 1:1 ligand:receptor, and suggest that upon binding, the ligand contacts two sites within the binding pocket, resulting in high affinity binding and receptor activation [8]. Also contributing greatly to our understanding of the ligand: receptor interaction has been a large number of studies using IGF and insulin analogues and species homologues [5,16,22,23].

1.3. A structure/function approach to understanding the IGF system

In the mid 1980s several groups, including Bayne, Cascieri et al. and Ballard et al., pioneered the use of IGF analogues to understand the mechanisms of interaction with binding partners. Since then many IGF analogues, including point mutants, IGFs with insertions or deletions and IGF/insulin and IGF-I/IGF-II chimeric ligands, have been used to gain a detailed understanding of molecular interactions controlling IGF action (reviewed in [5]). For example, early work exploited differences in sequence between insulin, IGF-I and IGF-II to identify residues important for IGF-specific interactions with the IGF-1R, IGFBPs and the IGF-2R [24–26]. In addition, we [27,28] and others [29] have produced a series of singly and multiply substituted IGF-I and IGF-II analogues to define the residues important for IGF-1R and IR-A (in the case of IGF-II) binding. Analysis of binding by these analogues led to the definition of two distinct binding sites on the ligands involved in receptor binding mentioned above. Furthermore, a series of IGF-II analogues were used to define the IGF-2R binding site and determine the basis for the binding specificity of this receptor for IGF-II [30,31]. A single amino acid, T16, is responsible for the binding specificity of the IGF-2R for IGF-II. When the corresponding residue of IGF-I (alanine) is substituted into IGF-II (i.e., T16A IGF-II) there is a complete loss of binding to the IGF-2R [30]. Further analogue studies

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