The Insulin Peptide Family

Insulin is only one member of a family of peptide hormones and growth factors that comprises 10 members in humans: insulin, insulin-like growth factors I and II (IGF-I and II) and seven peptides related to relaxin (Fig. 1). They result from successive duplications of an ancestral gene that appeared early in animal evolution. Invertebrates also have many insulin-like peptides, e.g. 37 in the worm C. elegans and seven in the fruit fly Drosophila Melanogaster[1]. They play an important role in metabolism, growth, reproduction and longevity.

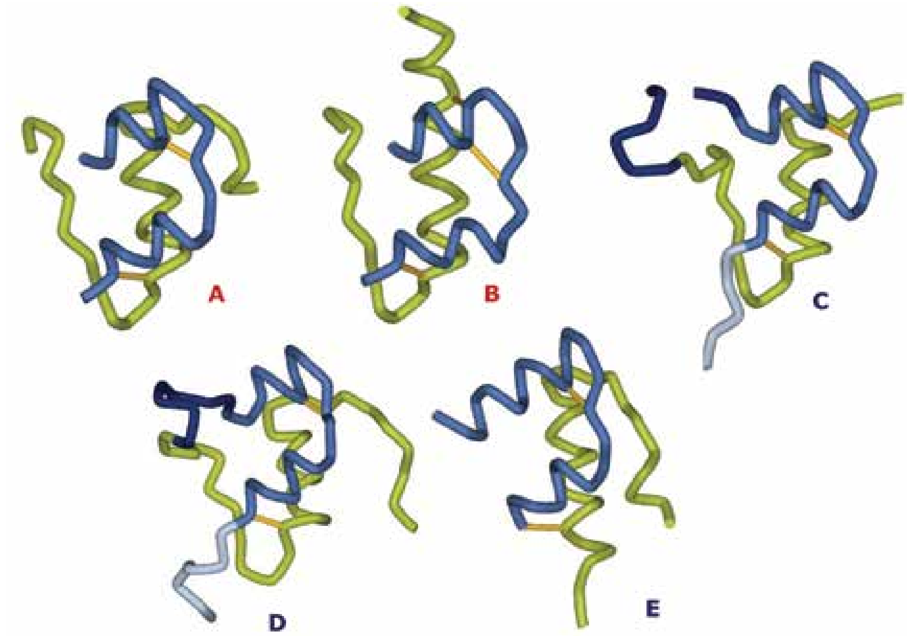

Figure 1: Canonical structure of members of the insulin peptide family. A. Insulin in the T conformation (PDB file 9INS). B. Insulin in the R conformation (PDB file 1ZNJ). C. IGF-I (PDB file 1GZR). D. IGF-II (PDB file 1ZNJ). E. Relaxin (PDB file 6RLX). The A-chains or A-domain are shown in blue, the B-chain or B-domain are shown in light green, the C-domain is shown in dark blue, and the E-domain is shown in light blue. The disulphide bridges are shown in yellow.

A Bit of History

Insulin was isolated and purified for the first time to a grade suitable for treating diabetic patients at the University of Toronto in 1921[2; see ref. 3 for a detailed history], winning the Nobel Prize in 1923 for Frederick G. Banting (1891-1941) and John J.R. McLeod (1876-1935). Besides being a life-saving therapy for diabetic patients, insulin turned out to be a bonanza for scientists interested in the structure and chemistry of proteins, and provided many technological milestones. Insulin was the first protein to be sequenced by Frederick Sanger (born 1918) in Cambridge[4], winning him his first Nobel Prize in Chemistry in 1958. It was the first protein to be made by total synthesis in the early 60s, independently by three groups: Panayotis Katsoyannis in New York[5], Helmut Zahn (1916-2004) in Aachen, Germany[6], and Yu Can Du and colleagues in Shanghai and Beijing[7]. It was the first protein to be assayed by radioimmunoassay, developed by Rosalyn S. Yalow (1921-2011) and Solomon A. Berson (1918-1972) in New York. This achievement won the Nobel Prize in Physiology and Medicine for Yalow in 1977. Human insulin became the first protein made by recombinant DNA technology (see section on insulin bioengineering) to be produced commercially in 1982[8].

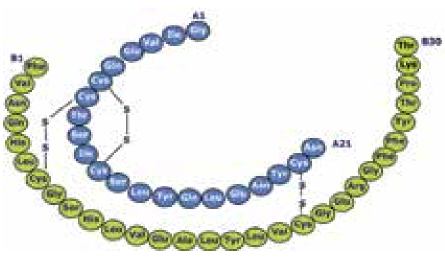

Figure 2: Primary structure of human insulin. The A-chain is shown in light blue, the B-chain in light green.

Insulin was first crystallised in 1926 by John J. Abel (1857-1938), Professor of Pharmacology at Johns Hopkins University in Baltimore[9]. Until the mid 60s, insulin crystals were improper for solving insulin's 3D structure by X-ray crystallography due to contaminants in insulin preparations. The efforts of Jørn Schlichtkrull at Novo at further purifying insulin resulted in monocomponent porcine insulin that went on the market in 1973[10]. Schlichtkrull provided Dorothy Crowfoot Hodgkin (1910 -1994) in Oxford (who had won the Nobel Prize for Chemistry in 1964 for the structure of penicillin and vitamin B12) with high-quality crystals, allowing her after over three decades of efforts to solve the 3D structure of insulin in 1969[11].

The insulin-like growth factors (IGFs) I and II were identified and sequenced in 1978 and found to be highly homologous to insulin. Their structure was solved in the 90s and early 2000s (see the section on discovery of the IGFs).

Relaxin was discovered in 1926 by Frederick Hisaw (1891-1972), Professor of Zoology at the University of Wisconsin and one of the founding fathers of endocrinology, as a factor from sow corpora lutea that caused the relaxation of the pubic symphysis of oestrogen-primed guinea-pigs (which was used as a bioassay for many years)[12]. Porcine relaxin was purified in 1950[13] and sequenced in 1977[14]. The sequence revealed unexpectedly (since relaxin does not have insulin-like effects) that relaxin is homologous to insulin. Methods for the solid-phase synthesis of relaxin were developed by Erika Büllesbach and Christian Schwabe at the Medical University of South Carolina, and John Wade and colleagues at the Howard Florey Institute in Melbourne, allowing detailed studies of the structure-function relationships of the hormone and related peptides[15]. The crystal structure of human relaxin was resolved in 1991 and revealed a fold similar to insulin and the IGFs (Fig. 1)[16]. Bioinformatics and

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genomic approaches identified seven sequences in the human genome coding for relaxin-like peptides: relaxin -1, -2 and -3 and insulin-like peptides (INSLs) 3, -4, -5 and -6. Surprisingly, unlike insulin and the IGFs which bind to receptor tyrosine kinases (see section on insulin and IGF-I receptors), the relaxins and INSLs bind to four separate G protein coupled receptors called RXFP-1 (also called LGR7), -2 (LGR8), -3 (GPCR135) and -4, with complex cross-reactivities. Further description of the properties of the relaxin subgroup of insulin-like peptides is beyond the scope of this article focused on insulin and cell culture; for further reading see ref 17.

Structure of Peptides of the Insulin Family

The circulating (and biologically active) form of insulin is a monomer (Fig. 1A) consisting of two chains, an A-chain of 21 amino acids and a B-chain of 30 amino acids (in man), linked by two disulfide bridges, A7-B7 and A20-B19. The A-chain contains an intra-chain disulfide bridge between A7 and A11[18,19]. At micromolar concentrations, insulin dimerises, and in the presence of zinc ions further associates into hexamers, a stunningly beautiful symmetrical structure (Fig. 2). In the 2-Zn hexamer

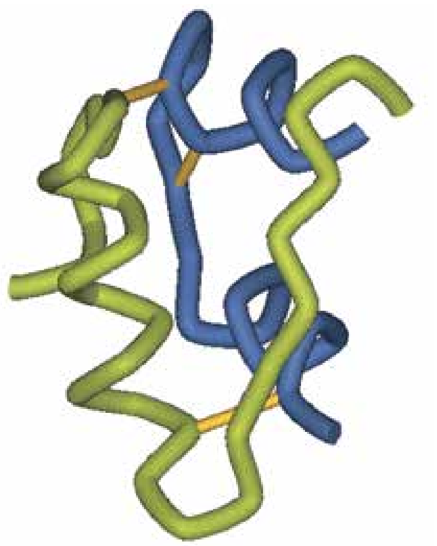

Figure 3: 3-D structure of the insulin monomer. Only the backbone is shown. The A-chain is blue, the B-chain is light green. PDB file 9INS.

solved by Dorothy Hodgkin and her colleagues, the A-chain has an N-terminal helix, A1-A8, linked to an antiparallel C-terminal helix, A12-A20. The B-chain has a central helix B8-B19, extended by N- and C-terminal strands. This crystallographic conformation is referred to as the T conformation (Fig. 1A)[20]. In the 2-Zn crystal form, all six monomers are in the T conformation (T6). An alternative conformation exists in which the B-chain helix extends all the way to the N-terminal (B1-B19, Fig. 1B), referred to as the R conformation. The canonical insulin fold described above (three helices, three conserved disulfide bridges) is present in all members of the insulin peptide family (Fig. 1) throughout evolution (see below) despite sometimes completely divergent sequences as in C. elegans.

Around its hydrophobic core, the insulin monomer has two extensive non-polar surfaces (Fig. 3)[18,19]. One is flat and mainly aromatic, and buried upon dimer formation in an antiparallel beta sheet structure. The other is more extensive and is buried when the dimers assemble to form hexamers. It is now clear that, not surprisingly for a small globular protein, insulin uses the same surfaces that it uses for self-assembly, for binding to its receptor[1] (see section on how insulin and the IGFs bind to their receptors).

Insulin is biosynthesised in the beta cells of the pancreas and stored in secretion granules as a precursor, proinsulin (Fig. 3), where the B- and A-chains are linked by a 35-amino acid connecting peptide. Proinsulin was discovered in 1967 by Donald F. Steiner (born 1930) at the University of Chicago[21-24]. Proinsulin is then cleaved at dibasic residues by a proteolytic system located within the

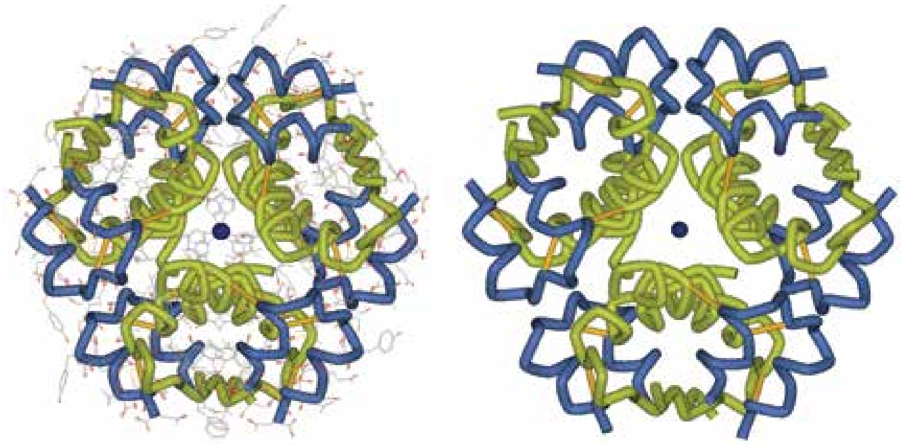

Figure 4: 3-D structure of the insulin hexamer.The structure of the R6 insulin hexamer is shown. The A-chains are blue, the B-chains (in the R conformation) are green. The two zinc atoms are shown in dark blue (the second one is hidden behind the first one), coordinated by the six His B10 histidines. PDB file 1ZNJ.

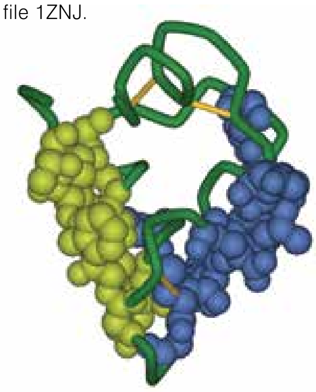

Figure 5: Insulin dimer- and hexamer-forming surfaces. The insulin surface involved in dimerization is shown in light green. It comprizes residues Gly B8, Ser B9, Val B12, Glu B13, Tyr B16, Gly B23, Phe B24, Phe B25, Tyr B26 and Thr B27. The insulin surface involved in hexamerization I shown in blue. It comprizes residues Phe B1, Val B2, Gln B4, Glu B13, Ala B14, Leu B17, Val b18, Cys B19, Gly B20, Leu A13, Tyr A14 and Glu A17. The backbone is shown in dark green. PDB file 9INS.

secretory granules of the beta cell. The C-peptide is co-stored with insulin in the granules and co-secreted with it in the bloodstream[23-24].

The seven mature relaxin/insulin-like peptides also have a two-chain disulfide-bonded structure (Fig. 1E), suggesting a similar biosynthetic process.

In contrast, IGF-I and IGF-II (Fig. 1C and D) are single chain peptides with a permanent C-domain of respectively 12 and 8 amino acids, that is contributing significantly to the IGFs binding to their receptors. In addition, a D-domain extends the A-chain C-terminus by respectively 8 and 6 amino acids in IGF-I and II.

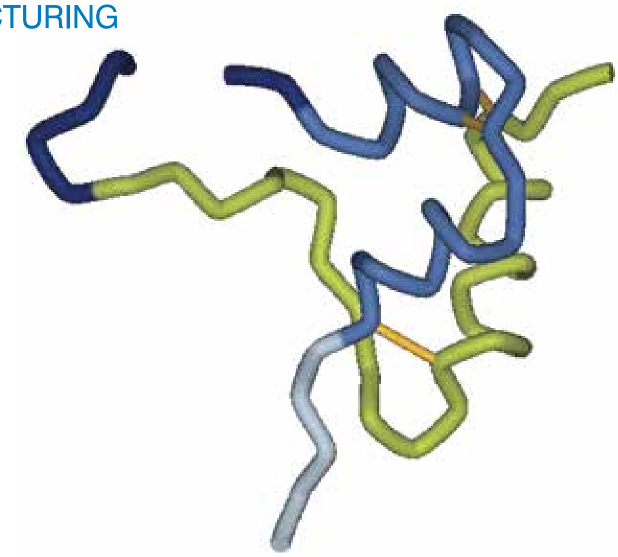

Figure 6: 3-D structure of the IGF-I monomer.Only the backbone is shown. The A-domain is green. The B-domain is blue. The C-domain is dark blue. The E-domain is light blue. The gap in the C-domain is due to the absence of Arg 36 and Arg 37 from the crystal structure. PDB file 1GZR.

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is an expert who has worked with and researched insulin and receptor binding for almost half a century. FeF Chemicals A/S is proud to present what we assume to be one of the best homepages describing the functionality of insulin and insulin like growth factors with respect to receptor binding and cellular response. More detail regarding Pierre de Meyts can be found at www.demeytsconsulting.com