The Insulin Active Surface Problem; 3D Studies on Insulin Analogues

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Insulin plays a key role in the regulation of free glucose in blood When the function of insulin is disturbed hyperglycemia can result, i e that the level of free glucose exceeds 1 2 g/l What is the role of insulin in this process? Insulin molecules are stored in b and a cells of the pancreas as complexes with zinc ions They are released into the blood in monomeric form Insulin monomers react with insulin receptors in the membrane Once insulin interacts with the receptor the receptor forms a dimer, and the dimer formation provokes autophosphorylation of the receptor The phosphorylated receptors interact with target proteins which become phosphorylated by the receptors and are thus activated These activated protein induce a cascade of processes which lead to the synthesis of glycogen Insulin consists of two chains, chain A and B The chains are connected between them by two disulfide bridges Besides these two disulfide bridges, there is an intra-chain bridge in the A chain

By crystallographic studies it was shown that insulin is always present in the crystals as a dimer (Dodson *et al* 1981) The dimer forms via an antiparallel β-sheet, where each molecule participates by one strand The dimerisation region in both molecules is the same, it involves the C terminal part of the B-chain, explicitly residues between B24 and B26 Depending on the crystallisation conditions, the insulin dimer is either symmetric or pseudo-symmetric The pseudo-dimers were observed in crystals of complexes of insulin with zinc ions (Dodson *et al* 1981) The symmetric dimers are found in the crystals grown in the presence of sodium salt (Gursky *et al* 1992) Biological studies have shown that the residues responsible for the dimer interaction are also the key residues responsible for the interaction of insulin with its receptor

We were interested in crystal studies of different insulin derivatives in which this C-terminal part of the B-chain responsible for the dimer formation was either deleted or modified These derivatives show different biological activities according to experiments realised in our laboratory For example des-octapeptide insulin (DOI) in which the last eight residues are absent, and which has biological activity close to zero The des-pentapetide insulin (DPI) which lacks the last five residues, contains a small part of the dimerisation motif and thus a small part of the molecule responsible for receptor interaction In the case of the OH termini DPI cannot dimerize and its measured biological activity is also close to zero In the case of the DPI derivative with an amino terminus, dimerisation is partially conserved and the measured biological activity is about 70% Besides these two derivatives semisynthetic analogues of DPI were prepared combining the replacement of the natural L-amino acid type by the D configuration with a methy lated peptide bond nitrogen on residues B24 and B25 These derivatives have biological activities which vary between 10% and 60% according to experiments realised in our laboratory

Initially, we crystallised the wild type insulin We found three different conditions Comparing the crystals forms, we supposed that we found two different crystals forms The crystals obtained according to the second conditions seemed to be different from the others two We collected data on this crystal on the beamline D41 of L U R E (France) The crystal is cubic, and it diffracted to the maximal resolution of 2Å R_{sym} of treated data was about 4% and their completeness at maximal resolution was about 93% The crystal measured is in fact analogues to the structure already deposited in the PDB (9INS –Glursky *et al*), and the structure was solved by the molecular replacement The refined model was compared with the PDB model 9INS, and we observed no significant differences at the backbone atom level However, we observed some differences on the side chain Generally, the differences are observed for residues, which in the original PDB model have double conformations

Table 1. The crystallisation conditions of insulin wild type, des-octapeptide$^{B23-B30}$ insulin (DOI) and Des-pentapeptide$^{B26-B30}$[N-Me)PheB25]insulin amide All crystallisation experiments used the vapour diffusion method

	INSULIN (WILD TYPE)			DOI	ME25-DPI
	Condition 1	Condition 2	Condition 3		
Protein	10mg/ml	10mg/ml	10mg/ml	10mg/ml	10mg/ml
Precipitate	30% PEG	1M Na acetate	20% 2-Propanol	25-30% Amonium Sulfate	1M Na acetate
Additives	0 2M Na Citrate		0 2M Na Citrate		
Buffer	0 1M Tris HCl, pH 8 5	0 1M Imidazol, pH 8 5	0 1M TES, pH 8	0 1M Tris-HCl, pH 8 5	0 1M Imidazol, pH 6 5

We obtained a crystal of DOI, using ammonium sulphate as precipitant, which have a needle form and they were very small They diffracted very poorly to a maximal resolution of about 6Å We crystallised also the derivative methylated on the nitrogen of peptide bond B25 of DPI This crystal diffracted well, to a maximal resolution about 2 5Å However, the unit cell cannot be determined for this crystal, which is probably due to imperfect growth from a heterogeneous protein solution, as detected by gel-filtration chromatography

References

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