Poster

Application of structural insights in the molecular engineering of insulin for recombinant expression in yeast

E. Johansson[1], G. Schluckebier[1], A. S. Andersen[1], F. Hubálek[1], F. F. Kreiner[1], P. Kurtzhals[1], T. Kjeldsen[1]

Novo Nordisk A/S

evjh@novonordisk.com

Insulin is a well-known and extensively studied hormone consisting of two peptide chains, the A-chain (21 amino acid residues) and the B-chain (30 amino acid residues). These chains are connected by two disulfide bridges and furthermore the A-chain contains an intrachain disulfide bond. In humans, insulin is produced in the beta cells of the pancreatic islets of Langerhans, with a 35-amino-acid-residue long C-peptide connecting the two chains. The C-peptide is removed upon insulin maturation.

The annual total need for pharmaceutical insulin is more than 40 tons. *Saccharomyces cerevisiae* (baker's yeast) is used to recombinantly produce half of the global requirement of insulin. In yeast, insulin is expressed as a precursor having an N-terminal extension as well as an engineered C-peptide connecting the insulin B-chain C-terminus with the N-terminus of the A-chain. This peptide is considerably shorter than the 35-amino-acid-residue long C-peptide described for the human insulin produced in the beta cells above. Importantly, the C-peptide needs to be systematically optimized to maximize the expression yield. After the secretory expression, the C-peptide is removed by enzymatic cleavage. Using the C-peptide as a removable structural element enables adaptions to different kinds of expressed insulins. This could for instance be differences in the oligomeric state of the insulin molecule, so that e.g., a fast-acting insulin with a monomeric behaviour requires a different C-peptide as compared to human insulin that forms dimers in solution.

Examples of insulin precursor design inspired by a crystal structure and the experimental structures of several different insulin precursors that improve the expression yields will be presented and used to explain the mechanisms. A review of this work has just been published [1].

[1] Kjeldsen, T., Andersen, A. S., Hubálek, F., Johansson, E., Kreiner, F. F., Schluckebier, G. & Kurtzhals, P. (2024). Trends in Biotechnology, 42, 464.