BIOTECHNOLOGICAL ROUTES FOR INSULIN PRODUCTION

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Abstract

Insulin is a peptide hormone produced by β-cells of the Islets of Langerhans in the pancreas and plays a central role in regulating blood glucose homeostasis. Historically, insulin therapy relied on extraction from animal pancreases, but this approach was limited by supply constraints, variable purity, and immunogenic reactions. The introduction of recombinant DNA technology in the late 20th century transformed insulin production by enabling biosynthesis in microorganisms such as *Escherichia coli* and *Saccharomyces cerevisiae*. These advances allowed the large-scale production of highly purified human insulin and eliminated dependence on animal sources.
This study reviews the evolution of insulin production methods, from animal-derived extraction to recombinant biosynthetic approaches, including the development of proinsulin-based systems and yeast expression platforms. Furthermore, it examines the structural and functional properties of insulin, its biosynthesis, receptor interactions, and metabolic effects on carbohydrates, lipids, and proteins. Special attention is given to insulin analogues, including rapid-acting (Humalog, NovoLog, Apidra) and long-acting (Lantus, Levemir) formulations, which were developed through targeted amino acid modifications to improve pharmacokinetic profiles and therapeutic outcomes.
Recombinant insulin and its analogues have significantly improved diabetes management by providing greater safety, consistency, and flexibility in treatment. Additionally, innovations such as insulin pens and advanced delivery systems have enhanced patient adherence. Overall, the development of recombinant insulin represents a major milestone in biotechnology and has fundamentally reshaped modern diabetes therapy.

*Keywords:*Insuline, yeast, gene modiffication

Introduction

Since the 1920s, insulin production relied heavily on large quantities of animal pancreases, primarily from cattle and pigs. Advances in chromatographic purification techniques during the 1970s enabled manufacturers to produce more purified insulin, thereby reducing the risk of developing anti-insulin antibodies, which had been shown to contribute to insulin resistance in a significant number of patients. However, these improved purification methods also required even greater amounts of glandular tissue per unit of insulin (IU). By 1981, the production of one kilogram of insulin required approximately 8,000 kg of pancreatic glands derived from around 23,500 animals—yet this quantity was sufficient to treat only about 750 diabetic patients for one year. Because insulin production was so heavily dependent on animal-derived glands, both supply and cost were closely tied to their availability (Zion Market Research, 2021*)*. During the 1970s, the supply of pancreatic glands in the United States declined significantly, while the prices of beef and pork fluctuated considerably, raising concerns about potential insulin shortages. These concerns were alleviated by a groundbreaking scientific advancement that utilized innovations in gene splicing developed in the mid-1970s. In 1978, scientists at City of Hope National Medical Center and Genentech developed a method for producing biosynthetic human insulin (BHI) using recombinant DNA (rDNA) technology *(FDA*). This multi-step process involved isolating and manipulating the two polypeptide chains (A and B) that constitute the human insulin gene, and then inserting them into a common bacterium, which was genetically engineered to produce large quantities of insulin. Eli Lilly and Company subsequently entered into an agreement with Genentech to commercialize human insulin using rDNA technology. On October 28, 1982, after only five months of review, the Food and Drug Administration approved **Humulin**, the first biosynthetic human insulin product and the first approved medical product of any kind derived from recombinant DNA technology (Ratner *et al,* 2000).The development of biosynthetic human insulin not only

addressed the instability of supply and pricing associated with animal-derived insulin, but also enabled the design of more efficient production protocols, achieving higher purity and improved standardization. Perhaps more importantly, the introduction of synthetic insulin marked a paradigm shift in diabetes therapy, paving the way for the development of non-animal-derived insulin products.These advancements led to the creation of insulin analogs with varying pharmacokinetic profiles, including rapid-acting analogs such as **Humalog** (approved by the FDA in 1996) and long-acting analogs such as **Lantus** (approved in 2000) (Hemmingsen *et al,*2021). This diversification of insulin products provided patients with a broader range of therapeutic options and greater flexibility in managing diabetes. From the 1980s onward, the insulin market increasingly focused on addressing both the clinical and lifestyle needs of patients with diabetes. This shift led to the introduction of innovations such as insulin pens and inhalable insulin formulations. While some of these products achieved greater commercial success than others, all were developed with the aim of improving patient adherence and enhancing the overall management of diabetes (Heinmann *et al,* 2000).

Material and methods

The data for this paper were collected from original and peer-reviewed scientific publications that can be found in online scientific libraries.The literature was searched based on the study topic, authors, and various journals. During the literature review, emphasis was placed on researching specific questions related to this topic. The literature was analyzed starting from general sources and progressing to articles directly related to this issue.The collected data were then corrected, organized, and compared with information obtained from the FDA.

Results

Insulin is a protein hormone. Its name originates from the Latin word *insula* ("island"), since it is secreted by groups of endocrine cells called the Islets of Langerhans in the pancreas.In general, insulin enables glucose metabolism by activating glycolysis, promoting the storage of glucose in the liver in the form of glycogen, and favoring fat storage. Insulin is a polypeptide hormone produced by the β-cells of the Islets of Langerhans in the pancreas, which play a role in regulating blood glucose levels (Mastick *et al,* 1998).
In addition to its effect on glucose metabolism, insulin also affects protein and lipid metabolism. Mitogenic activity has been observed at high insulin concentrations *in vitro,* which is considered to be mediated by insulin-like growth factor 1 (IGF-1). The main stimulus for insulin secretion from the pancreas is elevated blood glucose levels. However, insulin is also involved in other metabolic processes, such as stimulating the transport of glucose, amino acids, potassium ions (K^+), and other nutrients into cells. Insulin can stimulate the dephosphorylation of several regulatory enzymes involved in major catabolic and anabolic processes. Furthermore, insulin can cause changes in the transcription levels of various genes, most of which encode metabolic enzymes (Schwartz *et al,* 1997).
Insulin is a small peptide composed of two chains:
The A chain, consisting of 21 amino acid residues
The B chain, consisting of 30 amino acid residues (Drejer *et al,* 1991)
The two peptide chains are connected by two disulfide bonds at positions A7–B7 and A20–B19, while the A chain also contains an intramolecular disulfide bond between residues A6 and A11.The overall charge of the insulin molecule results from the ionization of acidic and basic functional groups of its amino acids. Insulin has an isoelectric point (pI) of 5.3 in its denatured state, meaning that at neutral pH it carries a negative charge. This property is important for insulin formulation development (Bornfeldt *et al,* 1991). Active insulin exists as

a dimer, but it is synthesized as a single molecule called preproinsulin, which is converted into proinsulin after leaving the endoplasmic reticulum. Proinsulin then undergoes proteolysis in secretory granules, producing active insulin and a connecting peptide (C-peptide), which consists of 34 amino acid residues.In the body, insulin is stored in pancreatic β-cells in the form of Zn-insulin hexamers, where it is stabilized by two zinc atoms. Each zinc ion is coordinated with histidine residues from three neighboring monomers (Rossana *et al*, 2010).

Insulin binds to the cell surface through a specific receptor. The insulin receptor is a tetrameric integral membrane glycoprotein composed of two α-subunits and two β-subunits. Binding of insulin to its receptor triggers the autophosphorylation of three specific tyrosine residues within the tyrosine kinase domain. The first method used to obtain insulin using recombinant DNA technology was from the company Genentech who consisted in inserting a gene encoding the synthesis of the A and B chains of insulin into *E. coli* cells (Owens *et al*, 2001). After culturing and growing the cells in large bioreactors, the product (either the A chain or the B chain separately) is purified using chromatographic methods. Furthermore, the two chains are incubated under oxidative conditions to form the disulfide bonds characteristic of insulin. Another example of DNA technology is the production of proinsulin who plays a crucial role in the insulin biosynthesis pathway, and its proper processing is essential for maintaining proper glucose homeostasis (Nidhi Sharma, 2022). The method of obtaining insulin with two separate chains was later modified by producing only one fused insulin–β-galactosidase protein, which can be cleaved in a single step to release mature insulin. This method consists of synthesizing proinsulin cDNA from mRNA by modifying its sequence through the addition of a codon at the 5^{th} position for methionine synthesis (Seo MJ, *et al* 2011). The modified cDNA is then inserted into plasmid genes and amplified in bacteria. The proinsulin produced in bacteria is subsequently separated from the β-galactosidase enzyme by degrading the methionine residue. The chain then tends to fold, allowing the formation of disulfide bridges, and the C-peptide is removed through an enzymatic reaction that produces the mature protein. Another metod was pthe production of insuline from yeast, Novo Nordisk S/A was the first company to produce proinsulin secreted by the yeast Saccharomyces cerevisiae (Son Y *et al*, 2007). To simplify the purification process, scientists decided to create a method in which insulin would be secreted directly into the culture medium after being produced inside the cell. For this reason, they used yeast instead of bacteria. Yeast is a eukaryote, meaning an organism that has a nucleus and is therefore capable of completing the maturation process of human insulin (Heinse T, 2021). The proinsulin sequence is inserted into a plasmid, and the recombinant plasmid is transformed into yeast. At this point, the yeast can produce proinsulin, which is processed in the same way as in humans. Proinsulin folds into a closed structure, and the disulfide bridges between cysteine residues can form normally. The 33-amino-acid pro-sequence is removed, releasing the mature protein. The development of recombinant technology enabled the production of different types of human insulin and its analogues (Thomas Kjeldsen *et al*, 2024):

Rapid-acting recombinant insulin- In the formulation of these insulins, modifications made to the amino acid sequence responsible for dimerization and oligomerization—namely, the substitution of these amino acids—ensure repulsion between molecules and prevent the formation of oligomers. Such insulins are absorbed 2–3 times faster after subcutaneous (s.c.) administration.

Humalog (insulin lispro)- is the first such preparation and is produced in *E. coli* through the proinsulin pathway. It differs from human insulin by the modified sequence LysB28–ProB29 (Buchbinder *et al*, 2000).

Novolog (insulin aspart) is another short-acting insulin analogue that differs in that the proline at position B28 is replaced by aspartic acid. With this modification, oligomer formation is reduced, ensuring a faster action.

Apidra (insulin glulisine) is an analogue with a modified amino acid sequence, in which aspartic acid at position B3 is replaced with lysine, and lysine at position B29 is replaced with glutamic acid, providing rapid action (Hemmingsen *et al*, 2021).
Long-acting recombinant insulin-the first registered preparation of this type is Lantus (insulin glargine and others), which has a structural modification (GlyA21, ArgB31, ArgB32). Specifically, in the A chain there is a substitution of asparagine at A21 with glycine, while in the B chain two arginine molecules are added. It is produced in *E. coli*.The modification of several amino acids contributes to increased stability and changes in the isoelectric point, so this insulin dissolves at acidic pH and is less soluble at neutral pH (in subcutaneous tissue). It is a clear solution with an acidic pH of 4.0 and contains zinc and m-cresol. After injection, it is immediately deposited in subcutaneous tissue, thereby providing prolonged action. Due to these properties, this insulin should not be mixed or diluted with other forms of insulin (Hemmingsen *et al*,2021. Levemir (insulin detemir) is another analogue of human insulin with a modified amino acid sequence LysB29(N-tetradecanoyl) and B30. (Hemmingsen *et al*,2021).

Discussion

The development of recombinant DNA technology marked a major advancement in the treatment of diabetes mellitus. Before the introduction of biosynthetic human insulin, insulin production depended mainly on animal pancreases obtained from cattle and pigs. Although animal-derived insulin was effective, its production faced several limitations, including limited availability of pancreatic tissue, unstable supply, high production costs, and the possibility of immune reactions caused by impurities. As the number of diabetic patients increased during the twentieth century, the need for a safer and more reliable method of insulin production became increasingly important (Mudaliar *et al,* 1999). The introduction of recombinant DNA technology in the late 1970s revolutionized insulin manufacturing. Genentech developed the first method for producing human insulin by inserting genes encoding the A and B chains of insulin into *E. coli* bacteria. This innovation demonstrated that human insulin could be produced on a large scale without relying on animal sources. The approval of Humulin by the FDA in 1982 represented a milestone in both biotechnology and diabetes therapy, as it became the first recombinant DNA-derived therapeutic product approved for clinical use.An important improvement in insulin production was the development of the proinsulin synthesis method. Instead of producing the A and B chains separately, scientists produced a single proinsulin molecule that could later be converted into mature insulin through enzymatic processing (Kurtzhals *et al*, 2021). This method simplified the manufacturing process, improved production efficiency, and enabled easier large-scale production. In addition, the correct folding of proinsulin and formation of disulfide bonds ensured the production of biologically active insulin similar to natural human insulin. Another advancement was the use of yeast, particularly *Saccharomyces cerevisiae*, in insulin production. Unlike bacteria, yeast is a eukaryotic organism capable of performing more complex protein processing. Novo Nordisk successfully developed a system in which proinsulin was secreted directly into the culture medium, simplifying purification and reducing production costs. The use of yeast further improved the quality and efficiency of recombinant insulin production. (Marlena *et al,* 2022) Recombinant DNA technology also enabled the development of insulin analogues with modified pharmacokinetic properties. Rapid-acting insulin analogues such as Humalog, NovoLog, and Apidra were designed to prevent insulin molecule aggregation, resulting in faster absorption after subcutaneous injection.(Gauarav *et al,* 2020) These analogues improved postprandial glucose control and provided patients with greater flexibility regarding meal timing. Long-acting insulin analogues such as Lantus and Levemir were developed to provide prolonged and stable insulin action. Structural modifications in these analogues altered their

solubility and absorption, allowing them to maintain blood glucose control over an extended period. These preparations reduced fluctuations in glucose levels and decreased the risk of hypoglycemia, especially during the night. The development of recombinant insulin and insulin analogues significantly improved diabetes management and patient quality of life. Modern insulin therapy allows more individualized treatment approaches adapted to patients' clinical and lifestyle needs. In addition, innovations such as insulin pens and inhalable insulin have contributed to improved treatment adherence and convenience. Despite these advancements, challenges still remain, particularly regarding the high cost of insulin products and their accessibility worldwide. Nevertheless, recombinant DNA technology has transformed insulin therapy by enabling the production of highly purified human insulin and advanced insulin analogues, greatly improving the safety and effectiveness of diabetes treatment. (Hemmingsen *et al*,2021).

Conclusion

The development of recombinant DNA technology revolutionized insulin production and significantly improved the treatment of diabetes mellitus. Before the introduction of recombinant insulin, production relied on animal pancreases, which created challenges related to limited supply, impurities, and the risk of immune reactions. The use of genetically engineered microorganisms such as *Escherichia coli* and *Saccharomyces cerevisiae* enabled the large-scale production of highly purified human insulin with greater safety and consistency. Furthermore, recombinant technology allowed the development of insulin analogues with different pharmacokinetic profiles, including rapid-acting and long-acting formulations. These innovations improved glycemic control, reduced the risk of hypoglycemia, and provided patients with greater flexibility in diabetes management. Recombinant insulin also reduced dependence on animal-derived products and opened new possibilities in biotechnology and pharmaceutical research. Overall, recombinant DNA technology has had a major impact on modern diabetes therapy and continues to contribute to advancements in patient care and treatment outcomes.

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