

Industrial Production of Insulin by Genetically Modified Organism

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Abstract - Insulin is a hormone secreted from the β cells of the islets of Langerhans, a specific group of cells in the pancreas. Life would not exist without insulin. In order to utilize the glucose (energy) found within the carbohydrate-laden foods we consume, insulin must be present to facilitate its (glucose) absorption. The rapid increase in the number of diabetic patients globally and exploration of alternate insulin delivery methods such as inhalation or oral route that rely on higher doses, is bound to escalate the demand for recombinant insulin in near future. Recombinant human insulin has been produced predominantly using *E. coli* (*Escherichia coli*) and *Saccharomyces cerevisiae* (a species of yeast) for therapeutic use in human. Current manufacturing technologies would be unable to meet the growing demand of affordable insulin due to limitation in production capacity and high production cost. Insulin is one of the most important drugs in medical science therefore it is important to have a basic knowledge about it. In this review we take a brief look on the history of insulin, its properties and function. We also focus on its industrial production concept and technique along with its purification process. We conclude with the current delivery system for insulin and future prospects.

Keywords: Insulin, Recombinant insulin, *E. coli*, Yeast, Industrial production

I. INTRODUCTION

Insulin medication is the use of insulin and similar proteins as a medication to treat diabetes. The genetics of diabetes has been an active area of research and development (investigation) for over the last 50 years. Since the early 1920s (discovery of insulin by Banting and Best in 1921), diabetic patients were treated with insulin, which was purified from bovine or porcine pancreas. The development in the field of genetic engineering allowed the production of insulin in *E. coli* and yeast. Genes encoding human insulin was cloned and expressed in *E. coli* in 1978. These developments paved way for the mass production of insulin at an economical cost to meet the demand of growing population. Throughout the world diabetes has reached an epidemic proportion [1], and therefore the role of insulin in treating diabetes, its resistance and its sequel is gaining prominence. Insulin is a key player in the control of intermediary metabolism. It has profound effects on carbohydrate metabolism by facilitating entry of glucose into muscle and tissues. Lipid metabolism also depends on insulin because it promotes the synthesis of fatty acids in the liver. Insulin also has ability to significantly influence protein and mineral metabolism [3]. Therefore abnormal

insulin level or responses have widespread and devastating effects on many organs and tissues [4][5]. Insulin comes in a number of different types including short acting such as regular insulin and long acting such as NPH insulin. It is used to treat a number of disease including diabetes and its acute complications such as diabetic ketoacidosis and hyperosmolar hyperglycemic states. It is also used along with glucose to treat high blood potassium levels [2]. Consequently, more than a century after scientists began to elucidate the role of the pancreas in diabetes, the study of insulin and insulin resistance remain in the forefront of medical research, relevant at all levels from bench to bedside and to public health policy.

II. HISTORY

Sir Edward Schafer who was professor of Physiology in Edinburgh is credited to name insulin and describe its actions. His work is compiled in the book called "Endocrine Organ" based on series of lecture he gave in California in 1913. Insulin was discovered 8 year later by Toronto surgeon Frederick Banting, assisted by a medical student Charles Best, under the supervision of John McLeod in May 1921. They injected the hormone into a diabetic dog and found that it effectively lowered the dog's blood glucose levels to normal. In 1923 Banting and MacLeod shared the Nobel Prize in Physiology and Medicine for the discovery of insulin. Insulin was isolated in the 1921 with its first clinical use in 1922 [6]. Since the early 1920s, diabetic patients were treated with insulin, which was purified from bovine or porcine pancreas. Genes encoding human insulin was cloned and expressed in *E. coli* in 1978. The first licensed drug produced using recombinant DNA technology was human insulin, which was developed by Genentech and licensed as well as marketed by Eli Lilly in 1982.

III. STRUCTURE AND FUNCTION

The human insulin comprises of 51 amino acids and has a molecular weight of 5808 Da. It is a simple protein consisting of two polypeptide chains, an A chain of 21 amino acids and a B chain of 30 amino acid, joined by two disulfide bridges in position 7 and 19. It is produced by beta cells of the pancreas and plays a key role in regulating carbohydrate and fat metabolism in the body. Pancreatic beta cell synthesizes insulin as a single polypeptide known as preproinsulin. Preproinsulin is converted into proinsulin

by signal peptidases, which remove signal peptide from its N-terminus. Finally, proinsulin is converted into the bioactive hormone insulin by removal of two basic pairs of amino acids, the C-peptide. Insulin is used along with glucose to treat high blood potassium levels [7] and was formerly used in a psychiatric treatment called insulin shock therapy [8]. It is also essential for stimulating liver to store glucose in the form of glycogen and also facilitates entry of glucose into muscle, adipose and several other tissues. Due to the long chain length, insulin is less soluble and has the ability to form micro-crystals when dissolved in water or milk. Insulin has now been proven to improve the health of the gut by increasing stool output, improving integrity of gut epithelium, and increasing absorption of minerals, significantly reducing the incidence of diarrhea, strengthening the immune system and stimulating growth of beneficial gut microbes [9].

IV. INDUSTRIAL PRODUCTION OF INSULIN

Concept

Amino acids are the basic units that build all proteins. The insulin gene code for a protein consisting of two separate chains of amino acids, A and B chain, that are held together by disulfide bonds. The insulin A chain consists of 21 amino acids and the B chain has 30. At first, preproinsulin is produced, which is a single long protein chain in which the A and B chains is not yet separated. A section in the middle links the chains together. There is a signal sequence at one end. Preproinsulin chain converts into proinsulin, which is still a single chain but there is no signaling sequence. A protein called active protein insulin starts its function to give insulin in a series of steps. At each step, this protein requires specific enzyme to produce the next form of insulin. Another popular method of manufacturing insulin is to grow the two insulin chains separately. This avoids the need of manufacturing each of the specific enzymes required in conventional method. Two mini-genes are required for this method: one that produces A chain and the other produces B chain. The exact DNA sequence of each chain is known and they synthesize each mini-gene's DNA in an amino acid sequencing machine. These two DNA molecules coding for chain A and B are inserted into small circular pieces of DNA known as plasmid. The plasmids are more readily taken up by the host's DNA. Plasmid is ready to be inserted into a non-harmful type of the bacterium *E. coli*. The recombinant, newly formed, plasmids are mixed up with the bacterial cells. Plasmids enter the bacteria in a process called transformation and the plasmid sticks to the bacterium's DNA by the process of recombination. It is inserted, next to the *lacZ* gene. *LacZ* gene codes for β -galactosidase which acts as selectable marker and helps in screening cells which contain insulin producing gene. It allows insulin to be produced in the cell, readily removed and prevent it from getting lost in the bacterium's DNA. Amino acid methionine is present adjacent to insulin gene in order to start the protein formation. The bacteria synthesizing the insulin undergo a fermentation process and

millions of bacteria replicate roughly every 20 minutes through cell division, each expressing the insulin gene. The cells are taken out of the reactors after replication and break open to extract the DNA. DNA is extracted by first adding a mixture of lysosome that digest the outer layer of the bacterial cell wall. A detergent mixture is added after that to separate the cell membrane. The bacterium's DNA is treated with a reagent called cyanogen bromide to splits protein chains at the methionine residues. This separates the insulin chains from rest of the DNA. The two protein chains are then mixed together and joined by disulfide bonds through the reduction-reoxidation reaction. An oxidizing agent (a material that causes oxidization or the transfer of an electron) is required to facilitate this reaction. Insulin batches are tested to ensure none of the bacteria's *E. coli* proteins are mixed with the insulin. *E. coli* protein is detected through a marker protein and is removed by different purification methods. At the end of the manufacturing process ingredients are added to the insulin to help maintain a neutral balance between acids and bases. An ingredient such as zinc oxide is added to purify insulin as to prolong its action.

Process

In industries insulin-making *E. coli* is grown in seed bioreactors, then scaled to manufacturing scale bioreactor as large as 50,000-liters. These are called fermentors and/or bioreactors interchangeably. Small seed tubes containing bacterial seed culture are stored in a cryogenic freezers (at -70 °C, -94 °F) in a controlled Working Cell Bank (WCB). For better safety in case of accidents the Master Cells are kept in a remote area called Master Cell Banks (MCB) to replenish the loss of the WCB stock. Due to low temperature storage environment the seeds in the tubes remain preserved and can be used decades later. To make a batch of insulin, an operator will go to the WCB freezer, pulls out a seed tube from the working cell bank and thaw out the content as to stimulate the bacteria to grow. The fresh culture starts with a mere half gram of bacterial seed, the microorganisms begin to replicate prodigiously, doubling their numbers every 20 minutes. Then the bacteria content transferred into larger and larger domiciles: from tube to flask to shaker to seed bioreactor and then finally to a manufacturing scale bioreactor. The microorganisms need media (water, sugar, salt, and other additives) and nitrogen to grow, which is supplied from a nutrient medium container/vessel. The *E. coli* are engineered to be resistant to a particular antibiotic, such as ampicillin. On adding ampicillin to a broth everything is killed off but the genetically modified protein producing bacteria. The bacteria are now ready to make insulin after several days of reproduction. Till this point, the bacteria have been kept from making insulin by a repressor protein that sits near the insulin gene. To start insulin production, the researchers add a chemical called an inducer to the giant vat of teeming bacteria in order to free up the insulin gene. The cells quickly start to produce insulin, holding the protein in clumps inside themselves. After a fixed period, typically a

few hours, it's time for the harvest and the hard work of isolating the insulin from mounds of bacterial debris begins.

Purification

The first step in the purification scheme is to separate the bacteria from the broth. That is done with an industrial centrifuge that settles the bacteria cells in the form of a pellet at the bottom of a vessel. The broth is then removed and replaced with a liquid containing a substance that breaks down the cell wall and cell membranes. It helps in the release of the insulin from bacterial cells. The insulin isolated in this way is actually not true insulin. It is "proinsulin," a longer inactive precursor of insulin. Insulin manufacturers use an enzyme to delete a section of proinsulin, leaving behind just the 51 amino acids of active insulin. The part that is snipped out is called C-peptide. It's a hormone, and it is a measure in the blood stream to check if the body is still making insulin in type 1 diabetic patients. The second step in purification involves a series of manufacturing scale columns made of a clear material like glass and packed with resin. The packed columns designed to separate insulin from other molecules based on differences in their electrical charge, acidity, basicity, size, and other characteristics. The insulin captured from the columns without any contamination or any other proteins. At the end of its journey through these series of columns, the insulin is quite pure. During processing, the amino acids chain of insulin gets tangled up, rendering it inactive. To get insulin into its proper form, manufacturers use another special mix of enzymes. The final step before the insulin is ready for packaging is crystallization. The insulin is mixed with zinc, which helps it to form stable crystals. The crystals are dried into powder of glistening crystals. The crystals can be rehydrated in solution and filled into vials, cartridges, and pens that are shipped around the globe.

V.DELIVERY MECHANISM

The traditional and most predictable method for the administration of insulin is by subcutaneous injections. This method is often painful and there have been reports of hypoglycemic episodes following multi dose injections of insulin [10]. Several new approaches to the method have been adopted to decrease the suffering of the diabetic patients including the use of supersonic injector, infusion pump, sharp needles and pens. Insulin that is administered by the jet injector method is absorbed rapidly without the risk of subcutaneous infection [11]. In mouth sprays, the insulin is absorbed through the inside of cheeks and in the back of mouth instead of lung [12]. Clinical experience has shown that inhaled insulin has the potential to be an effective treatment in patients with diabetes, with particular utility in the treatment of postprandial hyperglycemia [13]. While some of them eased the pain encountered by the diabetic patients, they offer incomplete convenience. Oral delivery offers the benefits of ease of administration, leading to greater acceptance by patients, improved absorption rates, and mimicry of the normal route of insulin

through the liver. The ultimate goal of the research in diabetics is to eliminate the need to deliver insulin exogenously and regaining the ability of patients to produce their own insulin. New concepts are currently explored to deliver insulin using patch, mouth spray, pills and inhalers. The success of the route of administration is judged on the basis of its ability to elicit effective and predictable lowering of blood glucose level and therefore minimizing the risk of diabetic complications. It is clear that several difficulties have to overcome with the use of formulation and application devices technology [16].

VI.FUTURE PROSPECT

The future of insulin holds many possibilities. Current manufacturing technologies will not be able to meet the growing demand of insulin due to limitation in production capacity and high production cost. Plant-based expression system holds tremendous potential for high-capacity production of insulin in very cost-effective manner. Recombinant human insulin has been successfully expressed and produced in oilseeds of plant *Arabidopsis thaliana* [14]. Since insulin was first synthesized, diabetics needed to regularly inject the liquid insulin with a syringe directly into their bloodstream. Researchers are exploring other drug-delivery options. Ingesting insulin through tablets is one possibility. Noninvasive transdermal insulin delivery could provide diabetic patients with sustained physiological levels of basal insulin in a pain-free manner [15]. Researchers are working on creating the pancreas cells that produce insulin in the laboratory. The thought is that physicians can someday replace the non-working pancreas cells with insulin-producing cells. Another hope for diabetics is gene therapy. Scientists are working on correcting the insulin gene's mutation so that the diabetic patient will be able to produce insulin on its own. The goal of the system is to have a single device that detect blood glucose levels and regulate blood sugar with insulin and other hormones without human intervention [17].

VII.CONCLUSION

Insulin has been termed as a boon to human kind. WHO has estimated that insulin sale would grow from \$12 billion to \$54 billion globally over the next 20 years. Dietary and lifestyle changes are causing dramatic increase in diabetes incidence all over the world and both Type I and Type II diabetic patients use insulin, however late stage Type II diabetes patients require large doses of insulin as they develop insulin resistance. Effective manufacturing and huge demand has made it one of the most distributed drugs throughout the world. There hasn't been much change in the manufacturing process of insulin since its industrial production started, though many companies are now producing different variety of insulin. The concept of industrial production of insulin remains the same with only few minor changes and therefore most of the research is now directed towards the delivery mechanism instead of manufacturing process. The demand of insulin has kept on

growing with the increase of the population confirming that insulin is one of the vital inventions of human kind.

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