Function and production of insulin

Insulin is a protein produced by islet cells in the pancreas. Insulin helps human body regulate glucose in the body. Insulin reduces the use of fats as energy (gluconeogenesis) by utilizing glucose to produce energy. Patient with diabetes require insulin to keep the blood glucose levels under control. (1)

The first successful insulin was produced from ox pancreas (bovine insulin). Bovine insulin is similar to human insulin however bovine chemical composition is slightly different hence the body immune system produces antibody to reject bovine insulin. (4)

Identical human insulin was produced by recombinant DNA technology, by inserting the insulin gene into a vector to produce human insulin. Production of human insulin by recombinant technology was accomplished and commercialized in 1982 by Genentech and Eli Lilly. (2)

Function

Carbohydrates consumed are broken down into glucose which then resides in the bloodstreams. This causes the body blood glucose (BBG) level to rise. The pancreas senses the rise in BBG level therefore, secretes insulin into the bloodstreams. The cell will absorb the released insulin and bind to it. Cells that are attached with insulin are able to absorb glucose from the bloodstream and turned it into energy. This energy is vital for new cell growth and repairing damage cells. The main function of insulin is to move glucose from the bloodstream into the body cells and convert glucose into energy. (3)

Chosen Host – Escherichia coli (E. coli)

E. coli was used as a host to produce recombinant insulin since 1982. E. coli was widely used as host for the production of recombinant insulin because it was well studied, many vectors available and good characteristic. (5)

E. coli advantages and suitability for producing insulin. (6)

Fast growth rate e. g. able to reach optimal yield within hours of induction.

Economical to grow and undemanding growth conditions.

Good protein production rate.

Can be frozen for storage and thawed immediately for usage.

Cellular structure

E. coli is a rod-shaped bacterium measuring 1. 8 microns in length and 0. 8 microns in width. E. coli consist of: (7)

Inner and outer cell membrane

Cell wall

Periplasm

Flagella

Pili

Cytoplasm

Chromosome

The two main structures are cytoplasm and periplasm, where the production of recombinant insulin usually takes place. (5)

Structure

Details and functions

Cytoplasm (7)

The cytoplasm contains most of the major component such as chromosomal DNA, RNA, nucleoid and ribosome.

Cytoplasm provides support for the internal structure and provides a medium of suspension.

Metabolic reaction and protein synthesis takes place in the cytoplasm.

Periplasm (7)

The periplasm is about 10nm thick.

Located between the inner and outer membrane.

Periplasm contains 80, 000 proteins essential for nutrient binding, enzyme detoxifying and degradative and electron transport.

Growth strategy for the production process

Insulin is produce through genetic engineering. Firstly the gene producing human insulin is isolated and copied. A circular shape DNA (plasmid) is

removed from the bacterium cell, and then using special proteins to cut open the plasmid ring. The insulin gene is inserted into the open plasmid ring and closed again using special proteins hence the human insulin gene is now combined with the bacterium DNA plasmid. The recombinant plasmid is then inserted into the bacteria cytoplasm using a very small needle syringe. (8) High level protein production often leads to formation of inclusion bodies which accumulated through protein folding. (5)

The chain approach method was used to produce human insulin in recombinant E. coli. Two different expression vectors were constructed carrying either insulin A- or B-chain gene fused to a shortened and inactive É£-interferon gene allowing a stable cytoplasmic production of recombinant insulin in E. coli in the form of inclusion bodies. (2) Recovery of biologically active insulin from inclusion bodies has some advantages for example inclusion bodies accumulates protein in the cytoplasm to a much higher level, inclusion body could initially be isolated in a purified and concentrated state with just centrifugation process and final insulin concentration in E. coli can be increased significantly by high cell density culture (HCDC). (5)

The HCDC technique was used to grow recombinant E. coli in a two stage cyclic fed batch bioreactor. After the expression system is developed, HCDC is carried out using synthetic medium with glucose as the sole carbon source to increase the recombinant protein concentration. (5) The induction of recombinant protein production was carried out by a temperature â€” shift from 30 to 42oC. Expressions of the A- and B-chain genes are controlled using a strong promoter (bacteriopage lambda) therefore, protein are produce efficiently by temperature shift. (2)

The first step of purification to obtain human insulin was to centrifuge the solution to isolate the inclusion body. The inclusion bodies were then recovered from the centrifuge continued by dissolving the inclusion bodies using formic acid. Then use cyanogens bromide to cleave the peptide bond by hydrolysing the peptide bond for separation of insulin from the fusion protein partner. Finally the conversion to human insulin is performed by proteolytic removal of the connecting C-peptide and disulfide link. (2)

The maximum productivity is achieved when the growth and production phase are separated hence a two stage cyclic fed batch bioreactor is used. The first stage is use to grow the cell to an optimum cell density followed by the second stage where the growth is suppressed by chemical and production of protein is maximize. (5) The cyclic fed batch is used to ensure that toxins and biomass do not accumulate, extend the productive phase, control the growth rate and optimize the product synthesis. (9) The by-product produced from the process was monomeric and multimeric forms of A- and B-chain connected by incorrect disulfide bridges. These by-products are collected too undergo sulfitolysis for recycling purposes. (2)

How to analyse the growth process

Off-line analysis methods were used to analyse the growth process. Samples were taken from the process every 30minute to be used to determine the optical density600, dry cell weight, dissolved oxygen tension and concentration of glucose. Result from the test are compiled and plotted in graphs therefore the growth process could be analysed. (2)

Possible problems and solution

The major problem in production of insulin by recombinant E. coli is the rapid intracellular degradation of the recombinant protein. Insulin is then produced as a fusion protein with a protein partner that would direct the recombinant gene product towards the formation of inclusion bodies. (2)

Proteolytic degradation and over expressing protein can destabilize the protein hence protein are produced in the form of inclusion bodies which are complicated and expensive denaturing and refolding process during the downstream processing. To overcome this problem, recombinant protein could be targeted at the periplasm instead of the cytoplasm hence avoided formation of inclusion body. (5)

In the process HCDC would cause several problems for example the limitation of dissolved oxygen due to high cell density and off gas accumulation which reduces the growth rate and enhance formation of acetate acid. The use of different promoters to regulate the level of expression and use of oxygen enriched air would minimize the problem. (5)

During the HCDC process, overproduction of recombinant proteins often results in cell filamentation and stagnant growth. The filamentation of cells consequently lowers productivity and final cell concentration. The problem could be overcome by suppressing the cell filamentation, by co-expressing the E. coli ftsA and ftsZ genes. (5)

Escherichia coli limitations

E. coli is not appropriate to produce large and complex proteins which contain disulfide bonds or protein that require post translation modification. (5)

Acetic acid is produce by E. coli when glucose is used as a carbon source. (5)

Secretion of protein by E. coli is a complex process often fails due to incomplete translocation across the membrane and insufficient capacity of the export machinery.(10)