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REVIEW ON INSULIN EXTRACTION AND PURIFICATION

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ABSTRACT

Insulin is a heterodimeric peptide linked by three disulfide bonds, and was first discovered in 1921. Insulin plays important roles not only in carbohydrate metabolism but also in anabolic regulation of proteins and lipids and regulation of the blood sugar level. This review further helps in to know about how to extract insulin as well as the purification of the insulin. And it's Role in the body.

KEYWORDS: Insulin, Purification, Glucose, Amino acid, Enzyme, E-coli, Recombinant DNA, Glucose.

1. INTRODUCTION

A. What is Insulin: The discovery of insulin in 1921 by Frederik Grant Banting and Charles Herbert Best,[1] when it was extracted from the pancreatic tissue of dogs.[2] Insulin is synthesized in the beta cells of the pancreatic islets of Langerhans. The secretion of insulin is controlled by the concentration of glucose in blood stream.[3] Insulin together with glucagon, control the blood glucose levels. Insulin concentrations increased as the level of glucose increases.

Insulin plays a major part in the uptake of glucose by the cells of the body. It stimulates the formation of glycogen in the muscles and in the liver, while suppressing gluconeogenesis by the liver. Insulin also controls the uptake of valine, leucine, and isoleucine by the muscles. Recombinant Human Insulin was developed by Genentech and marketed by Eli Lilly and company; in 1981 the FDA granted a marketing license to Eli Lilly and Co., for recombinant human insulin to be used medically in the US, the UK, West Germany and Netherlands. It became the first human hormone obtained in this way to be used to treat human disease.

B. Different types of insulin?

- **Onset:** Is defined as the length of time before insulin hits your bloodstream and begins to lower blood glucose.
- **Peak:** Is the time during which insulin is at its maximum effectiveness at lowering your blood glucose levels.
- **Duration:** Is the length of time insulin continues to lower your blood glucose levels.
- **Rapid-acting:** Insulin begins to affect blood glucose approximately 15 minutes after injection. It peaks in about an hour, and then continues to work for a few more.
- **Short-acting:** Insulin reaches your bloodstream within 30 minutes of injection. It peaks in the 2-3-hour range and stays effective for 3-6 hours.
- **Intermediate-acting:** Helps control glucose for 10-12 hours. A protamine is a type of protein that slows the action of this insulin.
- **Long-acting:** Insulin enters the bloodstream 1-2 hours after injection and may be effective for as long as 24 hours. Works more like typical pancreatic insulin**.**[6]

2. **Structure of insulin:** The structure of insulin is different among different species of animals. However, essentially it is a protein chain that is similar in many ways among animals.

Human insulin is closest in structure and function with cow (bovine) or pig (porcine) insulin. Bovine insulin differs from human in only three amino acid residues, and porcine insulin in one.

Normal insulin that is biologically active is monomeric or exists as a single molecule. The structure of insulin has 51 amino acids and 6000 Da molecular weight in almost all species, including human.[7] The human insulin molecule consists of two polypeptide chains, one A chain and one B chain containing 21 and 30 amino acid residues, respectively.[8] The amino acids of the two chains also participate in many non-covalent interactions.[7]

Two disulfide bridges (residues A7 to B7, and A20 to B19) covalently connect the chains, and chain A contains an internal disulfide bridge (residues A6 to A11). These joints are similar in all mammalian forms of insulin.

When secreted insulin joins in those to form dimmers and then in six to form hexamers.
This combination takes place in the presence of zinc.

The peptide chains then form 2 dimensional and three-dimensional forms. Each of these 3-dimensional structures have three helices and three conserved disulfide bridges. This is a basic fold. This basic fold is present in all members of the insulin peptide family.

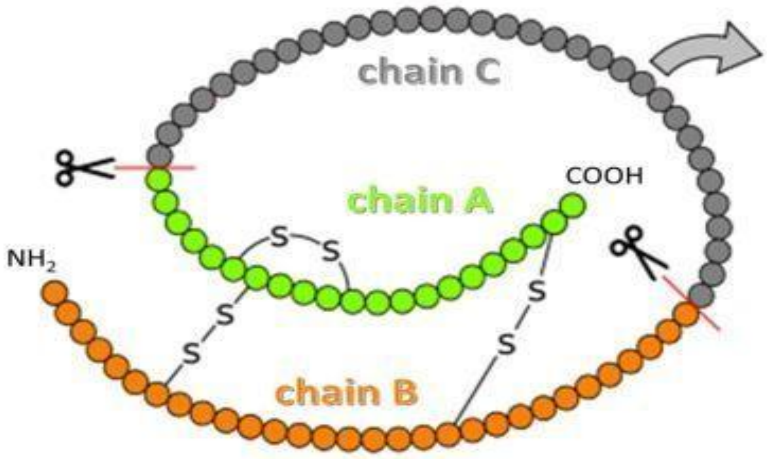

Fig. no. 1: Structure of insulin.

3. Role of insulin in the body

Insulin is a hormone produced by the pancreas that has a number of important functions in the human body, particularly in the control of blood glucose levels and preventing hyperglycemia, Insulin also has an effect on several other areas of the body, including the synthesis of lipids and regulation of enzymatic activity.

A. The main effects of insulin are

1) In the liver, to stimulate glucose oxidation and storage of glucose (glycogenesis), as well as to convert glucose into triglycerides and protein synthesis.
2) In the muscle tissue, it provides glucose uptake into the cells, and be stored as glycogen.
3) And in fat tissue, it provides glucose uptake and conversion to triglycerides for storage.[9,10]

The most important role of insulin in the human body is its interaction with glucose to allow the cells of the body to use glucose as energy. The pancreas usually produces more insulin in response to a spike in blood sugar levels, as occurs after eating a meal, for example. This is because insulin acts as a “key” to open up the cells in the body to allow for glucose to be used as an energy source.

Additionally, when there is excess glucose in the bloodstream, which is a condition known as hyperglycemia, insulin encourages the storage of glucose as glycogen in the liver, muscle, and fat cells. These stores can then be used at a later date when energy requirements are higher. As a result of this, there is less insulin in the bloodstream, and normal blood glucose levels are restored.

Insulin stimulates the synthesis of glycogen in the liver; however, when the liver is saturated with glycogen, an alternative pathway takes over. This involves the uptake of additional glucose into adipose tissue, leading to the synthesis of lipoproteins.[11]

B. Other functions of insulin

In addition to the regulation of glucose, insulin also plays a role in other areas of the body. To this end, insulin may be involved in:

- Modifying the activity of enzymes and the resulting reactions in the body.
- Building muscle following sickness or injury via the transportation of amino acids to the muscle tissue, which is required to repair muscular damage and increase size and strength. It helps to regulate the uptake of amino acids, DNA replication, and the synthesis of proteins.
- Managing the synthesis of lipids by uptake into fat cells, which are converted to triglycerides.
- Managing breakdown of protein and lipids due to changes in fat cells.
- Uptake of amino acids and potassium into the cells that cannot take place in the absence of insulin.
- Managing the excretion of sodium and fluid volume in the urine.[12,13]

C. What happens if I have too much insulin?

If a person accidentally injects more insulin than required, e.g., because they expend more energy or eat less food than they anticipated, cells will take in too much glucose from the blood.

This leads to abnormally low blood glucose levels (called hypoglycemia). The body reacts to hypoglycemia by releasing stored glucose from the liver in an attempt to bring the levels back to normal. Low glucose levels in the blood can make a person feel ill.

D. What happens if I have too little insulin?

People with diabetes have problems either making insulin, how that insulin works or both. The main two types of diabetes are type 1 and type 2 diabetes, although there are other more uncommon types.

People with type 1 diabetes produce very little or no insulin at all. This condition is caused when the beta cells that make insulin have been destroyed by antibodies (these are usually substances released by the body to fight against infections), hence they are unable to produce insulin.

4. Insulin\ Humulin production

Recombinant DNA used to produce human insulin

For many years, insulin was extracted and purified from either porcine or bovine pancreases, and this carried with it two main difficulties. The first was that though the animal insulin was chemically similar to human insulin, there were some differences, and these differences led to antibody attack and inactivation as well as inflammation in many patients. Also, there was the problem that this method of extracting insulin from animal organs made it difficult to obtain large amounts of pure insulin.

A. The steps in the production of human insulin by genetic engineering method includes

1. Human insulin is extracted from pancreas cells and an insulin-producing gene is isolated.
2. A plasmid DNA is extracted from a bacterium and cut with restriction enzyme, forming plasmid vector.
3. Insert human insulin-producing gene into the bacterial plasmid vector to form the recombinant DNA of human insulin-producing gene.
4. Introduce this recombinant DNA into a bacterial cell to form the recombinant bacterium.
5. The recombinant bacteria multiply in a fermentation tank and produce human insulin.
6. Insulin is extracted, purified and bottled. It is then ready to be injected into diabetic patients.[14]

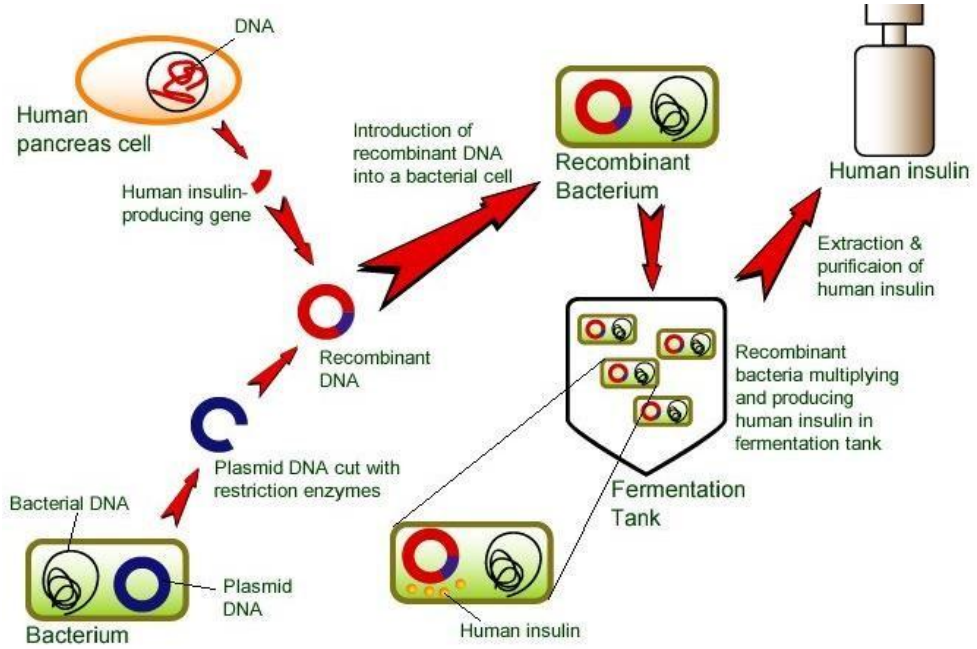

Fig. no. 2: Recombinant DNA used to produce human insulin.

B. Why E. coli is the best for insulin production

Escherichia coli is the preferred organism for insulin production for many reasons.

1. *E. coli* has the fastest reproduction rate which under the right conditions can double its numbers every 20-30 minutes.
2. It is also resistant to antibiotics such as ampicillin and tetracycline which allows insulin manufactures to easily inhibit the growth of unwanted microbes when it is fermented on a large scale.
3. *E. coli* is easy to handle which makes it very cost efficient to maintain. *E. coli* also produces the highest yields of insulin comparted to other organisms used for its production. All of this makes the production of insulin using *E. coli* the most profitable for manufactures.[15]

C. The production process of insulin using E. coli

Recombinant human insulin production using *Escherichia coli* begins with taking the insulin secreting cell from the human pancreas. From that cell the mRNA transcript is taken out to isolate the insulin human gene.

This will be done for both the A-chain protein and B-chain protein insulin forming genes that will be combined near the end of the production to form the complete insulin molecule.

The enzyme reverse transcriptase is attached to the mRNA which creates a single strand of cDNA. The cDNA is then polymerized by DNA polymerase to form a double strand of DNA.

That double stranded DNA is then multiplied by the polymerase chain reaction (PCR) which rapidly makes many copies of the DNA. At this point the DNA strand needs to be place in the plasmid of the *E. coli* cell.[16]

The *E. coli* plasmid has two antibiotic resistant genes one for tetracycline and the other is an ampicillin resistant gene. The restriction enzyme cutting point is in the middle of the tetracycline resistant gene which is where the plasmid opens to allow the human insulin gene to be inserted.

The gaps between the insulin gene and the rest of the plasmid are sealed with DNA ligase to form a complete recombinant plasmid. Since the restriction enzyme cutting point is in the middle of the tetracycline resistant gene once the plasmid is cut and the insulin gene is inserted the recombinant plasmid is no longer tetracycline resistant.[15]

The next step would be to insert the plasmids back into the *E. coli* cells. This is done by placing the cells into calcium chloride to make the cell membranes permeable and the plasmids are added to the mixture.

To identify which *E. coli* cells have taken up the recombinant plasmids manufactures us antibiotic resistance to distinguish between the four possible outcomes by adding ampicillin and tetracycline.

The cells that have a plasmid without the insulin gene would be resistant to both antibiotics. The cells that did not uptake any plasmids are sensitive to both antibiotics.[17,18]

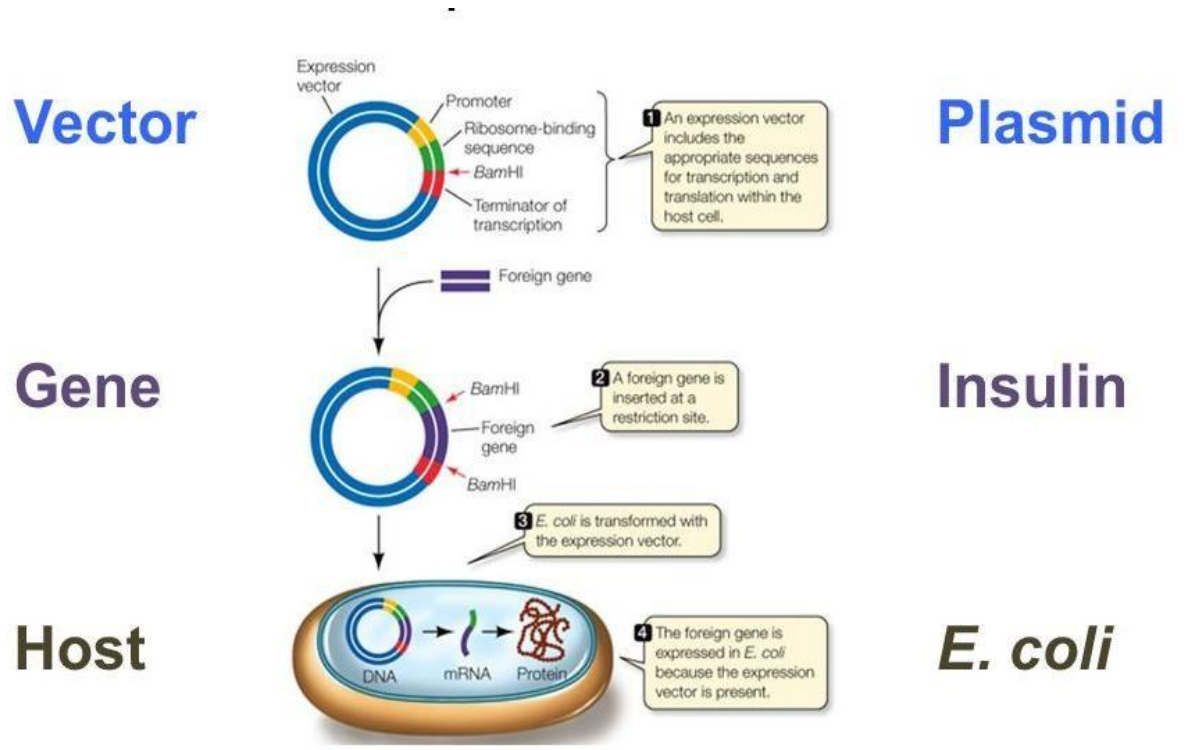

Figure 2: Requirement for recombinant protein production.

5. Purification of insulin

In the development of a purification process for insulin, a number of targets must be met. For a product such as insulin to be injected daily, the purity, measured by reverse-phase high-performance liquid chromatography (RP-HPLC), must be very high, and the content of host cell proteins and DNA must be very low.

The production takes place on a large scale because the world consumption of insulin is on the order of tons per year. Therefore, the purification processes need to be performed at a very large scale. Also, the purification process must be adapted to the actual fermentation process.[19]

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The purification process of human insulin by recombinant DNA technology is shown in Figure 3.

After transpeptidation, the crude insulin ester is purified by RP-HPLC at low ph. The purification step is performed on octadecyl-substituted silica, and the eluent is an ethanolcontaining ammonium sulfate buffer at pH 3.0.

After chromatography, the human insulin ester is isolated by crystallization. This step removes host cell proteins and trypsin as well as some by-products formed during the transpeptidation reaction. To keep trypsin inactive during the purification step, it is essential that the processing be performed under acid conditions. The purity of the resulting product is typically 90–92%, as measured by RP-HPLC.

After collection of the elute from the column, the product is isolated by crystallization, typically resulting in more than 94% purity, as measured by RP-HPLC.

Subsequently, in order to remove the ester group, the human insulin ester is subjected to hydrolysis, resulting in the formation of human insulin.

After hydrolysis the human insulin must be purified to comply with the specifications for the bulk product. The product contains several impurities such as small amounts of human insulin ester, partially cleaved precursor components, single-chain human insulin, and deamidated insulin.

Reverse-phase chromatography at low pH, traditionally used for the purification of peptides, does not suffice to ensure the required purity.[21]

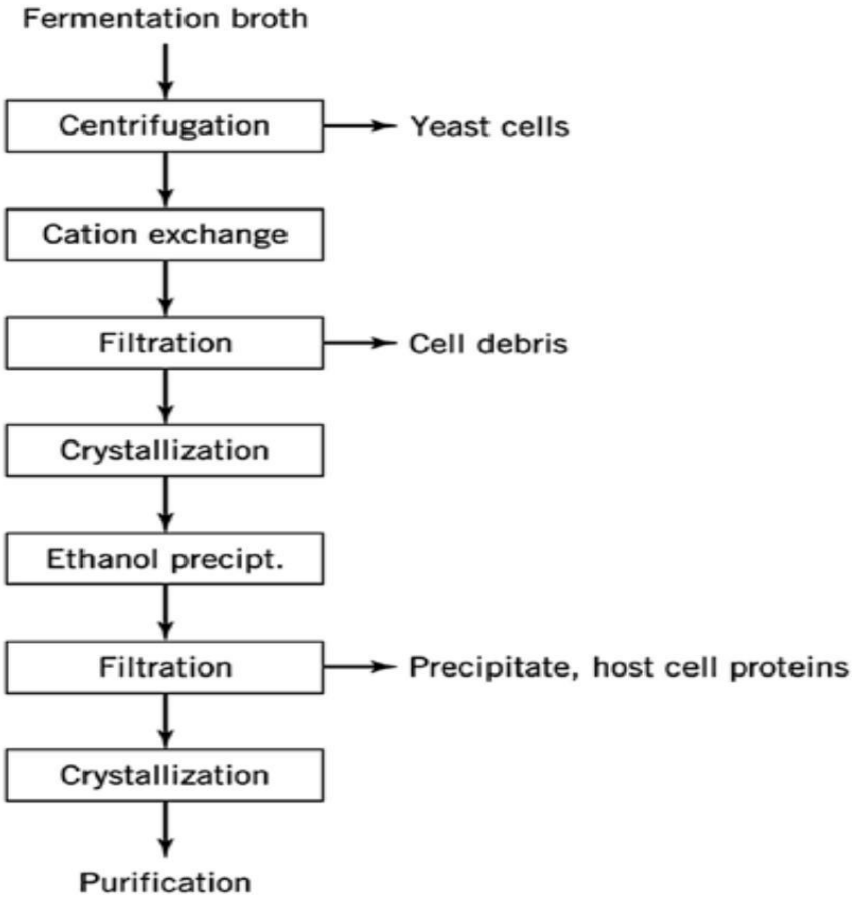

Fig. no. 3: Purification process of insulin.

6. CONCLUSION

Diabetes is a major threat to the human health. Hence the most effective method to treat diabetes is still insulin delivery thus, we have to knowledge of insulin. In this project, we study that it was able to extract, purified and formulate insulin from E-Coli. From the above review study we get easy and clear information about structure of insulin, role in body as well as insulin production and purification process. And the percent of diabetes patients in the world is increase to around 11% in next 10 years. This population can get knowledge about Insulin By visiting our Project.

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