ISSN: 2320-9267

Indian Journal of Pharmaceutical and Biological Research (IJPBR)

Journal homepage: www.ijpbr.in

REVIEW ARTICLE

The Use of *Escherichia coli* for Recombinant Human Insulin Production

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ABSTRACT

Insulin is the essential hormone produced by the pancreas which is accountable for sanctioning glucose we acquire from our food sources to be deposited in our body cells. Without insulin, our bodies cannot control blood sugar levels, so insulin is a vital hormone for survival. A diabetic person either does not produce insulin or is resilient to it for a multiple reasons. Because of this, they need insulin injections to process glucose. It has become stress-free for patients around the world to acquire insulin with the production of recombinant human insulin produced by *Escherichia coli*. This short review will provide an overview of the steps engaged in constructing recombinant human insulin utilizing the K12 strain of *E. coli* along with the prominence of recombinant insulin and why *E. coli* is most commonly used for insulin production.

Keywords: Insulin, recombinant human insulin, *E. coli*, mini review, biotechnology

Indian J. Pharm. Biol. Res. (2023): https://doi.org/10.30750/ijpbr.11.1.02

INTRODUCTION

The aggregation of diabetes incidence is increasing globally, leading to an escalation in insulin demand. According to the Centre for Disease Control and Prevention, more than 100 million Americans are diabetics or prediabetics.[1] The pancreas produces insulin hormones that control blood glucose levels.

A diabetic patient's first access to recombinant human insulin derived from *Escherichia coli* was in 1982, when it was available to diabetic patients for the first time.[2] Formerly it was collected from domestic animals like cows and pigs, which resulted in a lot of allergic reactions in patients.

Through the insertion of the human insulin gene into a plasmid from the *E. coli* bacteria, insulin could be translated and this breakthrough resulted in it to be was able to become available to patients as a more affordable drug. The purpose of this review is to explore why *E. coli* is the favoured organism for the production of recombinant human insulin.

Why *E. coli* is the preeminent organism in production of Insulin

The advancement in the field of genetic engineering permitted the production of insulin from *E. coli* and yeast, which have been accepted for therapeutic treatments in human by U.S. Food and Drug Administration.[4-5] Due to its fast reproduction rate and ability to double its population every 20-30 minutes under favourable conditions, *E. coli* is the favoured organism for insulin production. It is also impervious to antibiotics including ampicillin and tetracycline, which enables insulin manufacturers to impede unwanted microbe growth on a great measure effortlessly. *E. coli* is also easy to manage, establishing it to be very cost-effective to uphold.[3] Additionally, *E. coli* produces higher insulin yields when compared with other organisms used to produce insulin, making it the most profitable option for companies.[6] Insulin precursors (IP) are shaped into inclusion bodies using an *E. coli* expression system, then refolded and solubilised to produce fully functional polypeptides.[7]

Prior to the use of *E. coli* in the manufacture of recombinant human insulin, diabetic patients were dependent on insulin that was collected. from the pancreases of pigs and cows. There was however a certain contrast between the insulin molecules from pigs and cows at the

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How to cite this article: Datta N. The Use of *Escherichia coli* for Recombinant Human Insulin Production. Indian J. Pharm. Biol. Res. 2023;11(1):1-3.

Source of support: Nil

Conflict of interest: None.

Received: 10/01/2023 **Revised:** 18/01/2023 **Accepted:** 02/02/2023 **Published:** 25/03/2023

insulin receptor binding site, although it was operative in maintaining the patient's blood sugar at a optimum level. At the C-terminus of B-chain in human insulin, there is a threonine amino acid, while in pig insulin it is an alanine amino acid. There were three substitutions in insulin obtained from cow pancreas, namely an alanine amino acid on the C-terminal of the B-chain. On the A-chain, valine amino acids are at position A10 and alanine is at position A8, contrasting human insulin, which has isoleucine at position A10 and threonine at position A8.[8] In the past, insulin derived from pigs and cows was enormously exclusive to produce. For example, it would take around two tons of pig pancreas to harvest 8 ounces of insulin. As a result, insulin was quite expensive, and not everyone with diabetes could meet the expense of it.

Saccharomyces cerevisiae, a yeast strain [3] is also used to produce insulin. Like *E. coli*, S cerevisiae is used to yield recombinant human insulin. A human insulin gene is injected into a plasmid in the S cerevisiae cell using similar methods as in case of *E. coli*. Yeast based expression system produce solvable IP which is injected into the culture medium.[9-10] *Saccharomyces cerevisiae* is the most favoured and principal yeast for large scale marketable production of insulin, however numerous other substitute yeast strains have been discovered for insulin production.[11-13] Besides, *E.coli* and yeast, mammalian cells, transgenic animals and plant expression systems are also engaged as a host for large-scale manufacture of recombinant insulin.[14-15] However it is disadvantageous to use S cerevisiae since recombinant insulin is produced at much fewer productive rate than *E. coli*.[16] For *E. coli*, the productivity rate is ~1085. mg/hr at 80 g/l of biomass[17] and for S cerevisiae, the productivity rate is~104 mg/1 hr at 5 g/l of biomass.[18]

The Structure of Insulin Hormone

The human insulin molecule consists of 51 amino acids and has a molecular weight of 5808 Da. It is formed by beta cells of islets of Langerhans in the pancreas and controls carbohydrate and fat metabolism and absorption in human body. The pancreatic beta cells produce insulin as a single polypeptide called preproinsulin. The nascent polypeptide is focused to the endoplasmic reticulum by a 24-residue signal peptide present in preproinsulin.[6] During the translocation of the polypeptide, the signal peptide is severed, causing in the formation of proinsulin. In the endoplasmic reticulum, the proinsulin is folded correctly with the formation of three disulphide. bonds.

Weiss M, Steiner DF, Philipson LH. Insulin Biosynthesis, Secretion, Structure, and Structure-Activity Relationships. [Updated 2014 Feb 1] [Figure 1]. In: Feingold KR, Anawalt B, Boyce A, et al., editors. Endotext [Internet].By using prohormone convertases (PC1 and PC2) and exoprotease carboxypeptidase E, the folded proinsulin is then transported to the trans-Golgi network where it is converted into active insulin. The endopeptidases severs at two positions, causing in the discharge of a fragment termed as C-peptide.[6] Upon maturation, the fashioned insulin contains an A-chain containing 21. amino acids and a B-chain containing 30 amino acids. Both polypeptide chains are connected by two disulphide bonds and the A-chain contains a intra disulphide bond within itself.[19-20]

The manufacture procedure of Insulin using *E. coli*

The process of producing recombinant human insulin using *E. coli* initiates with the extraction of mRNA transcripts from insulin-secreting cells in the pancreas, in order to sequester the insulin human gene. For both A-chain protein insulin and B-chain protein insulin, this will be repeated. Both A-chain protein genes and B-chain protein genes will be used to produce the whole insulin molecule, which

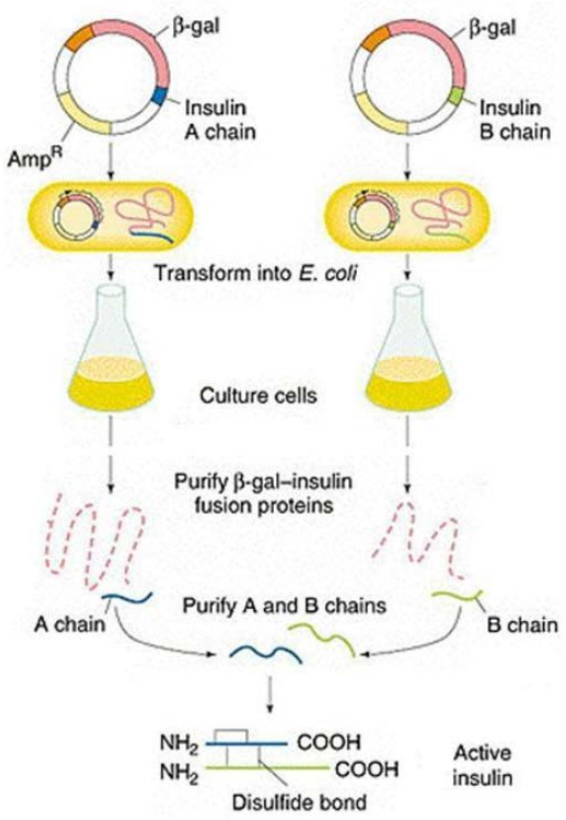

Figure 1: Primary structural sequence o insulin as determined by Sanger and co-workers

will be united near the end of production. A single strand of cDNA is formed by reverse transcriptase enzyme on the extracted mRNA, which is then polymerized by DNA polymerase into a double strand of DNA. In the next step, polymerase chain reaction (PCR) is used to increase the number of double stranded DNA, which creates a huge number of copies of it promptly. After this the DNA strand formed needs to be implanted in the plasmid of the *E. coli* K12 cell.[8]

In the *E. coli* plasmid, there are two antibiotic-resistant genes: a tetracycline-resistant gene and an ampicillin-resistant gene. A restriction enzyme (primarily EcoRI) enzyme) severs the plasmid at the tetracycline resistant gene, which allows the human insulin gene to be implanted into the plasmid. In order to form the whole recombinant plasmid, DNA ligase enzyme is used to close the gaps between the insulin gene and the rest of the plasmid. Upon severing and inserting the insulin gene, the recombinant plasmid is no longer tetracycline resistant since the restriction enzyme cutting point is in the middle of the tetracycline resistant gene.[6] *E. coli* cells is re-injected back with plasmids as the subsequent step. The cells are placed in calcium chloride in order to make their membranes permeable, and the plasmids are added to the combination. The cells are either heat shocked or electroporated to. enable them to absorb the plasmids. There are four consequences after the *E. coli* cells have taken the plasmids inside them. They include cells that took up the plasmids without insulin genes, cells that up-took no plasmids at all, cells that took up insulin genes without plasmids, and cells that took up the favoured recombinant plasmids. By adding ampicillin and tetracycline, we can discriminate between the four conceivable consequences by identifying which *E. coli* cells have taken up the recombinant plasmids.

The cells that have a plasmid excluding the insulin gene would be resilient to both antibiotics. The cells that did not uptake any plasmids are sensitive to both the antibiotics. Due to the restriction enzyme severing point being in the middle of the tetracycline resistant gene, the cells with recombinant plasmids would be resilient to ampicillin but sensitive to tetracycline.

Figure 2 gives a pictorial depiction of how the human insulin gene is implanted into the plasmid which is then introduced into the *E. coli* cell.[21] It is important that once the recombinant *E. coli* cells are recognised and secluded, they are then relocated to enormous fermenters where they will mature. In the broth, nutrients such as nitrogen, sugar, salt, and water are provided to the *E. coli* cells to ensure that they grow well. The broth is also infused with ampicillin

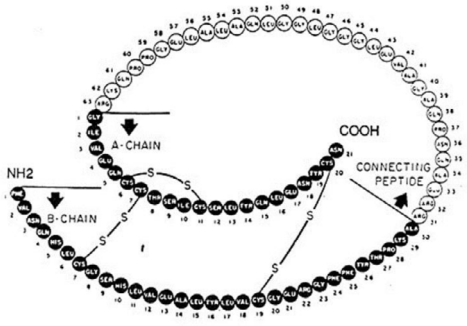

Figure after © 2000 by Griffiths *et al*

Figure 2: Recombinant Insulin Manufacture Procedure in *E.Coli* Host

to kill any microbes that have found their way into the fermentation tanks excluding the wanted ampicillin resistant *E. coli*, which reproduce every 20–30 minutes until their exponential growth population reaches saturation point.

It takes several days for the *E. coli* cells to reach a certain concentration when they are allowed to reproduce. Up to this point, the cells have been repressed from producing insulin due to the presence of the repressor protein near the insulin gene. In order for the cells to produce sufficient insulin, a chemical is added. In a matter of hours, the cells will have produced sufficient yield of insulin.[22] After collecting the cells from the fermentation tank, they are centrifuged to isolate them from the broth. Once the cells are detached from the broth, a chemical is added to break the cell membrane and release insulin. There are numerous purification steps involved in the production of the synthesised insulin prior to the 21 amino acid A-chains and the 30 amino acid B-chains are assorted together and joined by disulphide bonds in a 1:1 ratio.[23] The insulin is then for a second time decontaminated preliminary to the concluding step which involves crystallization. This is completed by the addition of zinc and desiccating the insulin to form into a crystal lattice before it is prepared to be packed and stockpiled for supply.

Importance of Recombinant Insulin

Many diabetics around the have benefited from the production of recombinant human insulin. Insulin is now more affordable, accessible and readily available than ever before, saving their lives. As a result, diabetics have been able to continue living their normal lives without having to worry about their blood sugar levels so much. As compared to animal and semisynthetic insulin, recombinant human insulin is more pure and pharmaceutically superior, and patients with diabetes can securely and efficaciously transfer

from animal or semisynthetic insulin to recombinant human insulin without altering their insulin dosage. The resolution for alteration remains a clinical objective, follow-up after any change of insulin product is suggested to confirm clinical efficiency. Furthermore, *E. coli* was discovered to produce other recombinant hormones indispensable for people with other diseases. For example, *E. coli* and other bacteria can also produce hormones to improve immune system function, infertility issues and blood production.[24]

Viewpoint of Recombinant Human Insulin Therapy

The customary care for diabetes has been recombinant human insulin for many years. A variability of appropriate arrangements are available like throwaway and reusable insulin pens for meal-time injection, basal support (Neutral Protamine Hagedorn) and conservative therapy. Due to accessibility and affordability, human insulin preparations are commonly used for type 1 diabetes therapeutics in many nations. There are numerous likely preliminary treatment options for type 2 diabetes, but insulin remains the key when glycaemic control cannot be attained without it. In many nations, compensation and pricing play a crucial role in treatment decisions.[25] It is imperative to reflect the economics of scale related with human insulin production and pharmaceutical dispensation of formulations. In many nations, human insulin and Neutral Protamine Hagedorn insulin are still the standard of care. In addition to rapid absorption and long period of action, insulin analogues deliver many assistances, with cost of treatment also being an imperative concern.[25] Efficacy of diabetes therapy with recombinant human insulin formulations. is well recognised, this is the major alarm for diabetes therapy in nations where affordability remains an imperative choice factor.

CONCLUSION

The fact that E. coli can produce so many aids such as recombinant human insulin is very surprising. This review has explained how the human insulin gene is positioned into the plasmid of *E. coli* cells and then how the insulin is taken out and processed. In addition to explaining why *E. coli* is the preferred organism for the production of recombinant insulin, the study also examined the control it has had on other recombinant hormones that are presently being produced. From being. derived from pigs and cows, insulin has evolved to what it is today. Considering recombinant human insulin formulations is increasingly influenced by access to affordable insulin therapy due to the widespread awareness that early insulin therapy can prevent or delay diabetes complication, it is possible to improve access to human insulin in developing countries in order to support initiatives .for earlier insulin therapy, improve community glycaemic control, and eventually delay or reduce diabetes complications for the long run.

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