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The Role of Recombinant DNA Technology in the Production of Insulin and Other Biopharmaceuticals

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Annotation: The invention of recombinant DNA technology provides a versatile way for generating proteins from cloned genes. Insulin was the first recombinant therapeutic protein widely used by millions of diabetes patients worldwide. Today, recombinant protein production continues to play a vital role in biopharmaceutical manufacture, providing at least 30% of the pharmaceuticals in the pipeline. It underpins the production of a large number of these drugs that have received regulatory approval and have become available worldwide for the treatment of a range of diseases.

At the beginning of the DNA era in the 1970s, scientists started to study the biology of DNA molecules in nature and also began to appreciate the tremendous additional utility of these molecules as vectors for assembling and transferring genetic information. In the 1970s, the main focus of biochemistry and molecular biology shifted from the

study of single molecules into the construction of molecules from parts, reflecting a belief in the understanding of life at a molecular level through the synthesis and analysis of biological components. The era of recombinant DNA technology was based on harnessing of naturally occurring DNA in cells to create standardized plasmids that could be used to construct new genetic entities with defined properties. Changes to biological targets are carried out through decoupling of the process of assembly and analysis into separate events.

Generating the fundamental recombinant DNA construct that can be used to produce the desired product at the industrial level requires considerable effort, and, normally, several iterations are necessary before the optimal clone is identified.

1. Introduction to Recombinant DNA Technology

Recombinant DNA technology is a sophisticated scientific approach that involves the establishment of genetic material that is derived from at least two distinct organisms. This highly specialized technique creates multiple new genetic combinations, which can then be transferred to a recipient organism in order to generate completely novel heritable material that may not naturally occur. These recombinant molecules play a crucial role as they are isolated and subsequently utilized to change and enhance the characteristics of various organisms. The genetic alterations brought forth by this technology may involve the precise insertion of fragments of genetic material taken from a wide array of different sources, and these fragments can be integrated into various types of vectors that serve as vehicles for the genetic material. In addition to that, recombinant DNA technology provides powerful tools for the manipulation of a genome. This can be achieved by either inserting specific DNA segments, which may lead to an increased expression of a specific gene product, or by blocking the expression of a certain gene, resulting in a reduction or even complete suppression of the production of that gene product. The generation of recombinant DNA involves the use of specialized enzymes known as restriction enzymes and DNA ligase. These enzymes are essential for preparing the recombinant molecules that are formed by the joining together of foreign DNA fragments with acceptor vector DNA molecules. After the recombinant molecules are successfully assembled, they are then introduced into host organisms where they are able to undergo replication and/or expression, ultimately resulting in the creation of a new gene product that can lead to advancements in various fields such as medicine, agriculture, and biotechnology. Through these processes, recombinant DNA technology enables scientists to explore the vast possibilities of genetic engineering and its applications for improving the characteristics of living organisms. [1][2]

2. Historical Background of Insulin Production

Before the advent of revolutionary recombinant DNA technology, insulin used for the treatment of diabetes was primarily extracted from the endocrine pancreas of various animals, including cows and pigs. This source of insulin was significant in the management of diabetes during that time. While animal insulin exhibited a similar three-dimensional structure to that of human insulin, it had notable differences in its amino acid composition. Despite animal-derived insulin being widely utilized for many decades in diabetes management, it could never fully replicate

human insulin. This discrepancy sometimes resulted in immunogenic complications for certain patients, adversely affecting the overall therapeutic effectiveness of the treatment provided. [3]

3. Mechanism of Recombinant DNA Technology

Recombinant DNA (r-DNA) technology is a set of techniques that are used to join together DNA molecules from two different species that are inserted into a host organism to produce new genetic combinations that are of value to science, medicine, agriculture and industry [1]. The basic mechanisms used in genome manipulation rely on the principal that two DNA strands will join together preferentially, if they have corresponding bases – adenine to thymine and guanine to cytosine. This can be modified by enzymes that cut and paste DNA in a very specific way. The isolating and cutting of DNA fragments involves restriction enzymes (endonucleases) and when already cut DNA fragments are joined together DNA ligase is employed. The joining of two DNA molecules gives rise to a hybrid molecule known as recombinant DNA. r-DNA comprises DNA from two different organisms. Multiple copies of a specific gene are involved and the basic process is to isolate the gene coding for the desired protein and place lo that gene in host organism capable of producing the protein in large quantities. Since the protein-coding gene is originated in another organism the technique is dubbed “recombinant DNA”.

One of the first successful applications of r-DNA technology was the production of human insulin using recombinant plasmids in a prokaryote host – Escherichia coli – a species of bacteria [4]. A plasmid is a small DNA molecule within a cell that is physically separated from chromosomal DNA and can replicate independently. Plasmids can be found in bacteria and in some eukaryotes. Similar to chromosomes, plasmids are double-stranded, typically circular DNA molecules. Recombinant plasmids, regarded as a fundamental tool for modern genetic, are used widely in molecular cloning, gene therapy research, and the large-scale manufacturing of recombinant proteins.

3.1. Gene Cloning

The two principal methods of recombinant DNA technology are gene cloning and polymerase chain reaction (PCR). Gene cloning involves the insertion of a DNA fragment of interest into a self-replicating genetic element, called a vector, that is then introduced into a suitable host and grown up under conditions that favour the production of a large number of copies of the recombinant DNA [1]. A typical cloning vector today is a small circular DNA molecule (a plasmid). There are many different plasmids available. Many also incorporate a selectable gene, such as one for antibiotic resistance, that allows the selective growth of host cells that contain the plasmid. A bacterial cell that has taken a plasmid up from its surroundings is said to be transformed. A DNA fragment of interest can be inserted into plasmids by exploiting the action of a group of enzymes called the restriction endonucleases. A number of different bacterial strains have been rendered transformation competent—and it is possible to greatly enhance the frequency of transformation by incubating bacterial cells in a CaCl 2 solution. The plasmids also contain a cleavage site for the restriction endonuclease used to cut the piece of DNA to be cloned, so that the two DNA fragments have ends that are complementary (“sticky ends”). These complementary ends can then be annealed and covalently joined using the enzyme DNA ligase [3]. The DNA fragment must first be excised from the source DNA by cutting with the appropriate restriction endonuclease, and then the cloning vector is cut with the same enzyme to produce complementary ends.

3.2. Transformation Techniques

Transformation techniques enable direct gene introduction into a new host, and can accommodate larger and more variable DNA sequences compared to transfection. Three common bacterial transformation methods are electroporation, conjugation, and chemical transformation. Electroporation applies brief electric pulses to cells, creating transient pores in cell membranes that permit DNA uptake. Conjugation, or bacterial mating, involves transfer of

DNA through cell-to-cell contact via a pilus, usually passing plasmids from a donor to a recipient. Chemical transformation uses divalent cations, such as calcium chloride, to neutralize cell surface charges, facilitating DNA entry into cells. Plasmids are readily introduced into bacterial strains through these methods, enabling protein expression. [5] [3] [1]

4. Production of Recombinant Insulin

Cell factories for insulin production Recombinant human insulin RHI has been produced mainly using either Escherichia coli or Saccharomyces cerevisiae Since insulin is the mostly prescribed medicine worldwide the demand for acquiring alternative methods of administration has also increased considerably thus the demand for the synthetic bionanomaterial recombinant insulin will escalate accordingly however the current technologies for manufacturing do not appear to be capable of meeting such a growing demand due to limited capacity together with labour healthcare and capital spending Consequently several approaches have been introduced with the purpose of increasing the amount of biologically active produced insulin among which the strategies based on transgenic plants seem to be particularly promising as they could provide high amounts of synthetic material in cost effective manners Moreover when targeted to seeds or leaves recombinant insulin expression levels can persistently achieve astonishing values while long term stability is also guaranteed thus providing a feasible strategy for both injectable and oral administration [3]

4.1. Selection of Host Cells

In the production of human insulin, the choice of appropriate host cells and expression systems for highly efficient and cost-effective high-level expression of the target product is important [5]. Both Escherichia coli and Pichia pastoris (yeast) are suitable hosts. E. coli is a preferred choice, while methylotrophic yeasts such as Pichia pastoris and Hansenula polymorpha can also be used [3].

E. coli is widely used for the heterologous production of various pharmaceutical proteins and industrially relevant enzymes and chemicals. Early attempts to produce human insulin by expressing the proinsulin precursor gene in E. coli were successful. Attempts were also made to express the two chains (A and B) of insulin in a single E. coli cell. However, this approach did not reach commercial-scale production. Humulin™ (Eli Lilly) was the first commercialized recombinant insulin product derived from genetically engineered E. coli.

The methylotrophic yeast Pichia pastoris offers attractive attributes as an alternative host system, such as rapid cell growth on defined media, scalable high-cell-density fermentation, the ability to perform post-translational modifications, and strong, tightly regulated methanol-inducible promoters. Investigation and optimization of the cultivation conditions at the bioreactor scale, accompanied by careful monitoring of physiological parameters, are essential to identify and control the parameters that affect biomass production, recombinant protein yield, folding, and other post-translational modifications. A high-yield recombinant expression platform was developed in a suitable mutant (Mut^s regulator strain), achieving a production of insulin precursor at about 3 g L^−1.

4.2. Expression Systems

S. cerevisiae has been employed for the production of recombinant biopharmaceuticals since the 1980 regulatory approval by the US Food and Drug Administration (FDA) of its first recombinant product, Humulin (a human insulin analogue) [6]. Recombinant protein expression systems include bacteria, yeasts, insect cells, filamentous fungi, mammalian cells, transgenic animals and plants, microalgae, and cell-free platforms. The choice of a production system depends on the target protein. Many proteins can be produced in prokaryotic systems, but recombinant proteins requiring post-translational modifications must be produced in eukaryotic systems, either single-cell systems or transgenic animals and plants. Systems able to perform post-translational modifications can deliver different glycosylation structures, a variance which

becomes critical when the biological activity of a recombinant protein depends heavily on glycosylation [3].

5. Purification Processes for Insulin

To obtain a pure insulin preparation, a combination of chromatography and filtration processes is employed. Multi-step chromatography is widely used to remove various contaminants and fractions with similar physicochemical properties, such as isoforms and misfolded variants. Polishing steps are necessary since affinity chromatography, while selective, cannot distinguish between closely related variants with similar physical and chemical characteristics. Typical techniques for polishing include hydrophobic interaction chromatography, anion exchange chromatography, and cation exchange chromatography. Commonly used fixed-bed chromatography columns include agarose, polymethacrylate, polyacrylate, ranges of ion exchange, and mixed-mode (e.g., hydroxyapatite, Capto adhere) resins. [3]

5.1. Chromatography Techniques

For recombinant protein production, the host gene is cloned and inserted into an expression vector. The resulting recombinant plasmid is introduced into an appropriate host. Expression of the target gene is induced through environmental or physical cues, such as changes in medium composition or temperature. The protein is then extracted and purified. Common purification techniques include chromatography and membrane-based processes [6]. Chromatography is widely applied to separately isolate and purify biomolecules and biopharmaceuticals. The sample is introduced on the top of a chromatographic column packed with a suitable stationary phase. The migration of compounds throughout the column bed depends on the flow rate of an added mobile phase (buffer or solvent) and on specific affinity, hydrophobicity, recognition, or size characteristics. Different types of chromatography are exploited according to the properties of the target molecule and the operational conditions.

5.2. Filtration Methods

Mechanical, biological, and physicochemical separation processes are widely utilized to concentrate, purify, and formulate the biopharmaceutical product while fostering production scheme design based on maximized mass transfer and enhanced fluid dynamics. Because biopharmaceutical solutions contain dispersed cellular components such as DNA, lipids, proteins, and organelles, downstream processing normally begins with a clarification stage consisting of centrifugation and filtration to eliminate larger particulate constituents. Biopharmaceuticals may be secreted or contained within host cells. Sequestered products are released by ultrasonic, high-pressure homogenization, enzymatic digestion, or chemical lysis. Dissolved gases can subsequently be removed by degasification. Throughout these steps bulk mRNA, host cell DNA, and proteins are largely removed, but additional clearance of smaller macromolecules and endotoxins by ultrafiltration and adsorption must be undertaken [3].

Chromatography modes include size-exclusion, hydrophobic interaction, ion exchange, and affinity operation. Chromatographic packing beds are fed in a continuous flow-through or column batch mode, or by periodic countercurrent or expanded bed operation. Methods may also be energy based in the form of electrophoresis, electrical field-flow fractionation, selective precipitation, or salting-out. Membrane filtration systems can be classified by the flow conducting media relative to the passive filtrate. Dead-end membranes, for example, operate in a single pass with feed flow oriented normal to the surface; filtrate passes through while retained solids accumulate and build a layer at the interface. In cross-flow membranes feed passes tangential to the membrane surface and solids remain suspended.

6. Quality Control in Biopharmaceutical Production

The development of an appropriate purification process is always based on a thorough knowledge of the target protein and trace components. Simultaneously, extensive analytical

methods may have to be developed so that the various process streams can be analysed accurately and quickly. In particular, the time needed for development of purification and analytical processes has often been underestimated, leading to delays in scale-up and commercialization.

A comprehensive and highly efficient assay program is critically required for accurate batch release as well as to ensure the safety and efficacy of the pharmaceutical products. Additionally, conducting drug substance and drug product stability studies is an essential and integral part of the overall quality control process. These stability studies play a crucial role in maintaining high-quality standards. Furthermore, it is essential that all analytical procedures are firmly established and meticulously followed to guarantee the utmost safety of the pharmaceutical product during both the production and storage periods, ensuring that the end consumer receives a product that is both safe and effective for use. [3]

6.1. Assay Development

Assay development constitutes a fundamental element in quality control, ensuring that biopharmaceutical products meet stringent efficacy and safety criteria. For insulin preparations, analytical methods have evolved from bioassays measuring biological activity to highly specific physico-chemical techniques. While bioassays originally correlated potency with glucose-lowering effects in rats or rabbits, they suffered from variability and limited reproducibility.

Contemporary regulatory standards mandate detailed evaluations of the active ingredient's type, amount, and bioactivity within the final therapeutic formulation. Consequently, advanced physico-chemical assays now predominate, frequently complemented by bioassays when immunochemical properties resemble a suite of impurities. Immunochemical assays, by capitalizing on antibody specificity, play a critical role in such contexts and also serve as the basis for potency assessments where relevant [5].

6.2. Stability Testing

Stability studies play a critical role in the quality assurance of pharmaceutical products, ensuring that they maintain their intended physical, chemical, microbiological, therapeutic, toxicological, and immunological safety and efficacy throughout product development and shelf life.

Ongoing stability testing during pre-approval clinical studies establishes appropriate shelf life and storage conditions on the final market formulation; stability testing continues after market distribution to ensure product characteristics remain unchanged during transit and storage. Shelf life therefore forms the accepted basis for moving batches throughout the supply chain.

A carefully designed, programmed, and controlled stability-testing program is therefore an integral part of quality control in pharmaceutical manufacture. Real-time studies complement accelerated studies, which expose the product to temperatures above normal. The International Conference on Harmonisation (ICH) Guidelines for stability testing provide the relevant criteria and conditions for both the formulation and packaging for which simulated tests can be performed. [3]

7. Regulatory Framework for Biopharmaceuticals

Recombinant DNA technology encompasses the deliberate forming of covalent bonds between DNA sequences to generate sequences that would not otherwise be found in the genome [3]. Insulin is a hormone essential for the metabolism of carbohydrates, fats, and proteins. Biopharmaceuticals are drugs also called therapeutic proteins, produced and extracted from genetically modified organisms. This novel technology has successfully revolutionized the health industry, allowing efficient and cost effective production of it, highly sensitive and specific diagnostic kits, vaccines and-by extension-effective gene therapy for hematological, genetic and hereditary diseases [1]. Prior to 1922, the lifespan of diabetic patients was very short, the discovery of insulin by Fredrick Grant Banting (1922) revolutionized treatment and served as a

milestone for recombinant DNA technology due to the difficulty in its purification from cattle or pigs' pancreas. Studies reveal that the discovery of restriction enzymes, able to cleave double stranded DNA at precise loci, has revolutionized gene cloning, which depends on the ligation of a DNA segment into vector DNA, followed by the introduction of vector DNA into a host cell. Rigorous regulatory standards have been placed on the development of recom-binant DNA pharmaceuticals to ensure that these products meet the highest standards for quality, safety and efficacy. Current regulations are based on the premise that recombinant DNA pharmaceuticals are "like" conventional therapeutic proteins, but they require more control because of the possibility that a "new" risk could be present. These new risks include, but are not limited to, the inadvertent incorporation of a gene or DNA sequence from a dele-terious virus or an oncogene into the product.

7.1. FDA Guidelines

Biopharmaceuticals derived by recombinant DNA technology must adhere to regulations issued by the United States Food and Drug Administration (FDA) under the Federal Food, Drug, and Cosmetic Act to assure safety and efficacy. Several guidelines have been established to acclimate international regulations with the diverse requirements of jurisdictions such as the United States, Europe, and Japan. These documents recommend the use of reference standards, validation of assay procedures, dose-response analysis, specificity assessments, stability assessments, and evaluation of various lot-to-lot products. Similarly, guidelines provided by the European Agency for Evaluation of Medical Products cover stability studies, analytical methods, and validations [3].

7.2. International Regulations

At the domestic level, only a few laws seem to regulate biopharmaceutical activities. Some countries refer to internationally recognized Quality Assurance Standards, such as the ISO 9000, which deals with quality management systems rather than safety issues. At the international level, several internationally recognized agreements have implications for the production of recombinant pharmaceuticals. Finally, important efforts have been undertaken to establish harmonized procedures for the registration and licensing of pharmaceuticals; these include the 3-CTD system that has been adopted by the European Union (EU), Japan, and the United States.

8. Applications of Recombinant DNA Technology

Recombinant DNA technology underpins the design and construction of new genetic materials, with widespread application in recombinant therapeutics. A growing repertoire of new products from this technology serves both as a replacement for deficient proteins and as treatments for various diseases, including antibiotics, hormones, and vaccines. In the clinical area, recombinant interferons and interleukins exemplify important therapeutic proteins introduced by this approach. Enzymes such as tissue plasminogen activator, used to dissolve thrombi in blood vessels, along with protein antagonists, offer further examples. Currently, recombinant biopharmaceuticals are defined as pharmaceutical preparations produced by recombinant gene expression in suitable host cells, encompassing purified proteins as well as monoclonal antibodies, antisense nucleotides, and gene therapy applications. These products, including well-known proteins like insulin and growth hormones, demand stringent quality, safety, and efficacy standards owing to their intended human use. This overview highlights key biopharmaceutical products and their biotechnological production processes [1].

In recent decades, the pharmaceutical industry has witnessed a steady increase in the development and approval of innovative biotherapeutic products. Many new drugs and vaccines utilize recombinant DNA technology to generate active pharmaceutical ingredients, thereby enhancing global health care of the nineteen recognized internationally available biopharmaceuticals [3].

8.1. Other Biopharmaceuticals

Biopharmaceuticals, a new class of drugs, are produced by recombinant DNA technology and are increasingly used worldwide. They are called biopharmaceuticals because they are manufactured by living cells or organisms instead of chemical synthesis. This represents the beginning of biological synthesis of drugs as opposed to chemical synthesis. Table 7 lists some important biopharmaceuticals. The market for biopharmaceuticals is growing rapidly and presently contributes nine percent of the total pharmaceutical market.

A number of new biopharmaceuticals are already under development. The future well-being of many AIDS patients appears to be roadblocked by a lack of suitable medicines and safe vaccines. However, the advancement in recombinant DNA technology is quite capable of meeting such current and future demands. [7][8][9]

8.2. Gene Therapy Applications

Gene therapy is an advanced technique with therapeutic potential in health services. Successful treatment of genetic diseases like adenosine deaminase-deficiency (ADA-SCID) has shown promising results, especially by targeting hematopoietic stem cells with improved gene transfer protocols. Gene transfer using lentiviral vectors has achieved success in treating X-linked adrenoleukodystrophy (X-ALD), indicating effective gene correction. Immunotherapy has been used to treat metastatic melanoma by enhancing specific protein expression, leading to tumor regression. Autologous T-cells modified to express Chimeric Antigen Receptors (CAR) targeting B-cell antigens have been employed in treating chronic lymphocytic leukemia. Combining gene and drug therapies has shown potential, such as conferring chemoprotection during chemotherapy for glioblastoma. Targeted gene transfer to specific sites has demonstrated impressive results in treating incurable autosomal recessive dystrophies like congenital blindness and Leber congenital amaurosis [2].

9. Ethical Considerations in Genetic Engineering

Genetic engineering often challenges the established values of society. Moral concerns arise regarding genetic manipulation of living organisms and transfer of genetic material between diverse species. Opponents fear discord between the genomes of host cells and recombinants and the possible consequences for the environment and evolution.

Genetic engineering enables production of material with the same bioactivity as human hormones, which may cause premature death if something goes wrong in the human body. Transgenic organisms are suspected of being among the causes of certain diseases. Authorities such as the Food and Drug Administration have established very precise regulations for the use of transgenic organisms and for the production of recombinant biopharmaceuticals. Despite these concerns, recombinant DNA technology is expected to generate at least 9% annual growth in biopharmaceutical production over the next five years [3].

10. Future Perspectives of Recombinant DNA Technology

Recombinant DNA technology is expected to facilitate production of a series of novel biopharmaceuticals, such as renin inhibitors, calcium channel blockers, antitumor agents, human serum albumin, heparin-binding epidermal-growth-factor-like growth factor (HB-EGF), and recombinant human hyaluronidase. Opposition to workers handling recombinant organisms has diminished; legislation for handling rDNA is becoming obsolete; and nearly all pharmaceutical discoveries of the next 25 years will depend on genetic recombination. Efforts focus on developing expression and safety systems and avoiding impurity escape. While bioinformatics has advanced, computational biology requires better tools. Genomic therapeutic control lacks an established basis for any gene, and application is distant. Stem-cell manipulation remains far from routine. The nineties will concern not with finding novel medicines, but with producing existing medicines efficiently and cost effectively. The interchangeable-factory concept of the

next decade should reduce time to market, technical risk, manufacturing cost, and capital investment by at least 10-fold. [10][11][12]

10.1. Innovations in Biomanufacturing

Over the last decade, biomanufacturing has experienced a transformation featuring the utilization of microbial factories for recombinant pharmaceuticals and integrated production of human insulin. Applications of recombinant DNA technology have also contributed to the production of next-generation insulin analogues and insulin derivatives. The biopharmaceutical market is growing due to increasing demand for novel biotherapeutics such as methotrexate, lepirudin, erythropoietin, and human growth hormones. With the advancement of recombinant DNA technology, the expression of active 5-enolpyruvylshikimate-3-phosphate synthase protein in Escherichia coli has been reported, as well as a thermostable cytochrome P450 (CYP450) BM3 4m mutant. Insights demonstrate that transgenic seeds can serve as warehouses for recombinant insulin [3].

10.2. Emerging Biopharmaceuticals

Recombinant insulin has paved the way for several other recombinant biopharmaceuticals, including human growth hormone, follicle-stimulating hormone, and clotting factors. Emerging biopharmaceuticals currently under research or at early stages of commercial development include cancer vaccines, diabetes mellitus treatments, cosmeceuticals, tissue engineering, and gene therapy. Pharmaceutical companies are exploring multivariate genome engineering and high-throughput screening technologies to enhance the development of recombinant biopharmaceuticals, with a projected global market value of $1,255.1 billion by 2024 [3].

11. Case Studies in Insulin Production

Eli Lilly developed the first commercial recombinant and the first commercial human insulin, Humulin, sold under the brand name Humulin. Novo Nordisk’s three marketed recombinant human insulins and one marketed recombinant human insulin analog are also described [5] [3].

11.1. Lilly's Humulin

Recombinant DNA technology is an exemplary innovation in biotechnology and is at the forefront of numerous biopharmaceuticals [1]. The technology combines genetic material from multiple sources to create novel DNA molecules. The molecules developed may be self-replicating and act as vectors or transmissible from one organism to another. Recombinant DNA technology permits the cloning of genes in a recombinant vector and several techniques determine its expression in various available host cells [3]. Insulin manufactured using recombinant DNA technology is the first commercial pharmaceutical product produced by gene cloning and recombinant DNA technology. Eli Lilly & Co. marketed the product as Humulin® in 1983. Several commercialized recombinant insulin products exist, supporting an evaluation of the human insulin landscape from a pharmaceutical company perspective.

11.2. Novo Nordisk's Insulin Products

Novo Nordisk began producing recombinant human insulin in 1982 and launched both human insulin and faster-acting insulin aspart products. Since introducing recombinant human insulin and insulin analogues, Novo Nordisk has expanded its portfolio to include over 20 injectable insulin products and delivery devices [3].

In modern biopharmaceutical manufacturing, quality-by-design approaches have led to development and optimisation of chemical and enzymatic processes for peptide tagging. The design of co-expressed proteases enables salt-free flow through purification, delivering tagless recombinant insulin precursors with high yield and purity. A straightforward three-step chromatographic pilot-scale purification method generates bioactive human insulin and insulin analogues. This first large-scale biotechnological-downstream process ensures high yield and quality over commercial-scale operation, efficiently yielding tagless insulin.

12. Impact of Recombinant Insulin on Diabetes Management

The advent of recombinant DNA technology has had a profound impact on the management of diabetes mellitus. Since the 1980s, mammalian gene products, including insulin, have been produced via recombinant DNA technology to treat various metabolic and cardiovascular diseases, cancer, and other genetic disorders. Recombinant insulin, indistinguishable from native human insulin, is now the drug of choice for these conditions [1].

The primary goal of recombinant insulin production is to supply large quantities of biochemically defined proteins for therapeutic use. Although insulin blends still constitute the most popular therapy for both type I and type II diabetes, scientists have sought better alternatives [3].

Earlier forms of insulin therapy, often derived from animal sources, were associated with several drawbacks, including allergic reactions and limited availability. Recombinant DNA technology has addressed these issues by facilitating the large-scale production of pure, bio-identical human insulin. Consequently, recombinant insulin has become widely accepted by patients, manufacturers, and physicians due to its established safety profile and improved therapeutic efficacy.

13. Challenges in Biopharmaceutical Production

Manufacturing biopharmaceutical proteins requires extensive capital investment. Although annually approved biopharmaceuticals and sales are growing, costs are enormously higher than production of small-molecule pharmaceuticals using synthetic chemistry [3]. These elevated production costs, which increase the return-on-investment requirements, represent a major challenge faced by many pharmaceutical companies. Likewise, entry costs present a formidable purchase price worth to protect the incumbent’s position against new entrants. Moreover, lack of large-scale, cost-effective, and noneggressive downstream purification processes for biopharmaceuticals currently forces the use of small, expensive facilities to perform purification.

Production scale-up remains a common and unresolved problem in the biopharmaceutical industry. Enhanced production capability for intricate and novel therapeutic proteins is urgently needed so as not to threaten the sustainability of the entire industry. For example, the production capacity of therapeutic crystallizable fragments (Fc) has not kept pace with the number of drug candidates employing this protein platform. Similarly, the production of regulatory proteins, including human growth hormone, erythropoietin, interferons, and tissue growth factors, lags far behind anticipated demand for these biopharmaceuticals. Due to problems related to cost and economies of scale, the production of pharmaceutical-grade plasmids also appears to be a major problem. Such issues present major challenges that currently impede the continued advance of recombinant DNA technology into new and more innovative types and sources of biopharmaceuticals.

13.1. Cost of Production

The rising worldwide demand for human insulin comes not only from the ever-increasing diabetic population but also from the necessity for administering higher doses through innovative delivery methods such as inhalation or oral routes. This growing demand exerts significant pressure on the need for cost-effective and large-scale manufacturing processes. Furthermore, the existing limitations of current bioprocessing technologies in terms of both capacity and cost-effectiveness make it incredibly challenging, if not impossible, to meet the ever-expanding global demand for insulin. As the requirements escalate, it becomes vital to address these manufacturing challenges to ensure adequate supply. [3]

13.2. Scalability Issues

Biopharmaceutical products are characterized by substantially higher production costs compared to small molecule biopharmaceuticals. Specifically, recombinant proteins can be up to 10 times

costlier, while monoclonal antibodies may reach 100 times the cost. Moreover, increasing process volumes may not proceed linearly; procedures like column chromatography and filtration scale sub-linearly. Insulin market growth is further dampened by the prevalence of generics, which is also a relevant consideration for other recombinant biopharmaceutical products.

Cost and scalability issues currently constitute major limiting factors to the expansion of the biopharmaceutical market. Mitigating these challenges is essential to support growth. For biopharmaceutical proteins, cell factories are presently the only available production platform [3].

14. Comparative Analysis of Conventional vs. Recombinant Insulin

Conventional insulin derived from porcine or bovine pancreases generally classifies as monocomponent preparations, while recombinant human insulin is a multicompound complex. Because of their differing composition, their efficacies differ; for example, the lipid profile in diabetic patients (especially total cholesterol and low-density lipoprotein cholesterol) improves significantly after treatment with recombinant human insulin rather than with porcine or bovine insulin. Substantial differences exist between purified porcine insulin and recombinant human insulin in the design of commercially available forms and pharmacokinetic profiles. The technological principle underlying human insulin production remains similar to that used for porcine insulin; however, additional techniques have been developed to improve production and are implemented in modern bioreactor systems. To date, three major technologies for commercial recombinant human insulin production have been developed: a strain of Escherichia coli expressing human proinsulin fused to a small carboxy-terminal peptide of β-galactosidase; another strain of E. coli expressing two separate chains of human insulin (A and B chains) that are refolded and oxidatively combined to form insulin; and a Saccharomyces cerevisiae strain containing a plasmid with the porcine insulin gene sequence, in which only three amino acids differ from human insulin. The remaining major challenge in the manufacturing of recombinant human insulin formulation is the high manufacturing cost and difficulty of large-scale production [3].

15. Patient Perspectives on Recombinant Insulin

Patient experiences have shaped recombinant insulin adoption. Early users appreciated its effectiveness and reduced hypersensitivity compared to animal-sourced insulin [3]. Patients also valued its human origin [1] , aligning product characteristics with therapeutic goals.

16. Global Market Trends for Biopharmaceuticals

Biopharmaceuticals have emerged as a remarkably fast-growing sector within the overarching pharmaceutical industry, with recombinant DNA technology playing a pivotal role as a major contributor to this impressive growth trajectory. As of 2017, the global market value of biopharmaceuticals stood at a substantial USD 160.5 billion, and it is projected to soar to an astonishing USD 526.1 billion by the year 2025, signifying a robust compound annual growth rate of 16.4% during the period from 2018 to 2025. Notably, biopharmaceuticals account for a significant 50% of the top-selling 100 drugs available worldwide, illustrating their critical importance and efficacy in modern medicine. Since the year 2008, there have been nearly 690 recombinant protein drugs introduced into the global pharmaceutical market, showcasing the innovation and advancements in this field. Current trends in the operations of regulatory authorities, coupled with breakthroughs in manufacturing technology and a growing market demand for newly developed and advanced biopharmaceutical products, are fostering a healthy and dynamic growth environment within the industry. Furthermore, the steady increase in the approval of biosimilars and biobetters, along with the impending expiration of several patents, creates opportunities for the integration and incorporation of various emerging technologies leading up to the year 2025. Additionally, emerging regions such as China, Korea, and India are

actively cultivating a bio-similar-friendly environment, which in turn is propelling the rapid expansion and development of the biopharmaceutical industry in these areas. This overall landscape indicates a promising future for biopharmaceuticals, with ample growth potential and a positive impact on global health. [3]

17. Conclusion

Recombinant DNA technology has emerged as an efficient, potent, and alternative-looking scientific approach in the medical field, facilitating novel therapeutic procedures and the development of new protein products. Its invention has expanded the horizon of biopharmaceutical drug development. Biopharmaceuticals encompass a collection of medicinal drugs produced through recombinant DNA technology, facilitating the production of biochemical substances that cannot be synthesized through chemical means. The integration of P450 cytochrome components with the insulin receptor enables the extensive synthesis of insulin, addressing the critical demand for this hormone. As biotechnology advances, the production of new and promising biopharmaceuticals will increase, impacting global markets and patient populations.

The application of recombinant DNA technology in biopharmaceuticals, both direct and indirect, offers precise methods for combating life-threatening diseases compared to traditional medicines. The development of advanced medications will improve quality of life and extend life expectancy. Beyond its therapeutic uses, the technology also provides solutions in forensic science, aiding in criminal identification and DNA profiling. Despite ongoing ethical debates, recombinant DNA technology has become an essential component of the modern pharmaceutical sector, underscoring the need to address related ethical concerns.

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