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PHARMACEUTICAL BIOTECHNOLOGY

UNIT 1

TOPIC :

- d) Brief introduction to Protein Engineering.



Protein Engineering

Protein engineering is a branch of biotechnology in which proteins are modified to improve their properties or to create new functions.

Proteins are altered using:

- Recombinant DNA technology
- Genetic engineering
- Chemical modification methods

The main aim of protein engineering is to obtain proteins with better activity, stability, specificity, and therapeutic value.

Proteins

Proteins are large biological molecules made up of amino acids.

The function of a protein depends upon:

- Number of amino acids
- Sequence of amino acids
- Three-dimensional structure

Even a small change in amino acid sequence can change the structure and function of the protein.

Levels of Protein Structure

Proteins are mainly divided into four structural levels:

1. Primary Structure
2. Secondary Structure
3. Tertiary Structure
4. Quaternary Structure

1. Primary Structure

→ The primary structure is the linear sequence of amino acids joined together by peptide bonds.

Importance

- Determines overall protein structure
- Any change in sequence may alter protein function

2. Secondary Structure

→ The secondary structure is the local folding of protein chains into specific patterns.

Types

- Alpha helix
- Beta pleated sheet

Stabilization

Maintained by hydrogen bonds.

3. Tertiary Structure

→ The tertiary structure is the three-dimensional arrangement of a single protein chain.

Stabilized By

- Hydrogen bonds
- Ionic bonds
- Hydrophobic interactions
- Disulfide bonds

Importance

Determines biological activity of protein.

4. Quaternary Structure

→ The quaternary structure is formed when two or more polypeptide chains combine together.

Example

- Hemoglobin

Concept of Protein Engineering

If the amino acid sequence of a protein is changed, the structure and function of the protein may also change.

Protein engineering involves intentional modification of proteins to make them:

- More stable
- More active
- More useful in medicine and industry

Need of Protein Engineering

Natural proteins are not always suitable for industrial or pharmaceutical applications because they may:

- Work only at specific temperatures
- Become unstable at high heat
- Be sensitive to pH changes
- Work slowly
- Act only on specific substrates

Therefore, proteins are modified to improve their performance.

Objectives of Protein Engineering

- Increase enzyme activity and efficiency
- Improve protein stability
- Increase resistance to heat and chemicals
- Improve enzyme specificity
- Produce better therapeutic proteins
- Reduce side effects
- Create proteins with new functions

Approaches of Protein Engineering

Two major approaches are:

1. Rational Design
2. Directed Evolution

1. Rational Design

- In rational design, scientists first study the structure and function of the protein.
- Specific amino acids are intentionally changed using genetic engineering techniques.

Features

- Planned and targeted changes
- Requires knowledge of protein structure
- Used to improve activity and specificity

Advantages

- Precise modification
- Predictable results

Disadvantages

- Requires detailed structural knowledge

2. Directed Evolution

- Directed evolution is a method in which random mutations are introduced into genes coding for proteins.
- Many protein variants are produced and screened to select the best one.

Features

- Mimics natural evolution
- No detailed structural knowledge required

Steps

1. Random mutation generation
2. Production of protein variants
3. Screening and selection of best protein

Advantages

- Effective for complex proteins

Disadvantages

- Time-consuming

Techniques of Protein Engineering

Main techniques include:

1. Site-Directed Mutagenesis
2. Protein Engineering by Gene Families
3. Protein Engineering through Chemical Modification

1. Site-Directed Mutagenesis

→ Site-directed mutagenesis is a molecular biology technique used to introduce a specific and intentional change in DNA sequence.

Principle

Change in DNA sequence → change in amino acid sequence → change in protein structure and function.

Basic Steps

1. Select target gene
2. Design primers containing mutation
3. Perform PCR amplification
4. Remove original DNA
5. Insert mutated DNA into host cells
6. Produce mutated protein

Applications

- Enzyme modification
- Drug development
- Research studies

2. Protein Engineering By Gene Families

→ This method uses similar genes from the same gene family to create improved proteins.

Mechanism

- Compare similar genes from different organisms
- Combine useful parts of genes
- Produce hybrid proteins with improved properties

Example

Development of heat-stable enzymes from thermophilic bacteria.

Advantages

- Produces proteins with combined beneficial properties
- Useful for industrial enzymes

3. Protein Engineering Through Chemical Modification

→ In this method, proteins are chemically modified using chemical reagents.

→ Specific chemical groups are added to proteins or amino acids are altered chemically.

Example

PEGylation:

Addition of polyethylene glycol (PEG) to proteins to improve stability and reduce degradation.

Advantages

- Improves stability
- Increases half-life of drugs
- Reduces immune reactions

Disadvantages

- May reduce biological activity
- Complex chemical process

Applications of Protein Engineering

1. Pharmaceutical Industry

- Production of recombinant insulin
- Development of monoclonal antibodies
- Vaccine production
- Therapeutic protein development

2. Industrial Enzymes

- Heat-stable enzymes
- Detergent enzymes
- Food processing enzymes

3. Medical Applications

- Gene therapy research
- Drug targeting
- Cancer treatment

4. Agricultural Applications

- Improved crop proteins
- Disease-resistant plants

Advantages of Protein Engineering

- Improves protein efficiency
- Enhances stability
- Reduces side effects
- Produces better medicines
- Useful in industries and healthcare

Disadvantages of Protein Engineering

- Expensive process
- Complex technology
- Ethical concerns
- Risk of unwanted mutations