Brief Introduction of Protein Engineering

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Introduction

Protein engineering is the process of developing useful or valuable proteins by modifying their amino acid sequences or structures.

It is a multidisciplinary field that combines biology, chemistry, and technology to create novel proteins for various applications.

The goal of protein engineering is to create proteins with new or improved properties, such as increased stability, enhanced activity, or new binding abilities.

Objectives

1. Increasing substrate affinity to enzyme
2. Makes the enzyme thermal tolerant (active at high temp) and P^H stable.
3. Enhances the substrate specificity by modifying the substrate binding site of the enzyme.
4. Designing the enzyme to make it resistant to proteolytic degradation.
5. Synthesizing enzyme that is stable and active in non-aqueous solvents.

Objectives

6. Changing the enzyme in order to make it independent of cofactor for its function.
7. Improving the stability of the enzyme to heavy metals.
8. Fusing the enzymes needed in the reactions to give a final product.
9. Produce hybrid enzymes.
10. Make isolation and purification of enzymes simpler.

Techniques

Techniques used for protein engineering fall in two basic categories

(1) Genetic modifications:

(i) Site directed mutagenesis

(ii) Localized random mutagenesis

(2) Chemical modifications:

(i) Change in functional group on side chain

(ii) Modification & replacement of original protein

Site Directed Mutagenesis

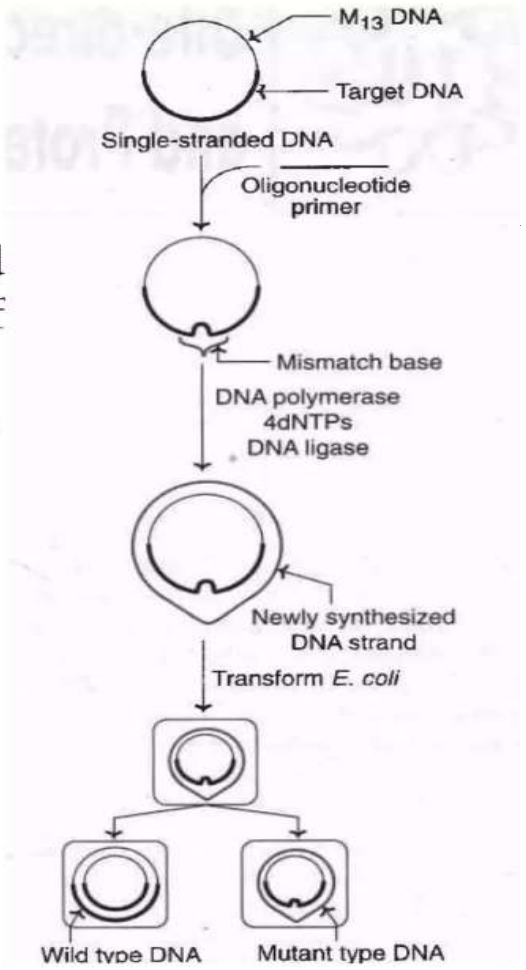
- Site directed mutagenesis is defined as a change in nucleic acid sequence (or genetic material) of an organism at a specific predetermined location.
- Site directed mutagenesis is the technique of generating amino acid coding changes in the DNA (gene). By this approach specific (site directed) changes (mutagenesis) can be made in the base of the gene to produce a desired enzyme.
- Directed mutagenesis can be done using:

M13 Plasmid DNA, PCR, Random Primer, Degenerate Primer, Nucleotide analogue

THE SINGLE PRIMER METHOD

In the technique of oligonucleotide-directed mutagenesis, the primer is a chemically synthesized oligonucleotide (7-20 nucleotides long).

- It is complementary to a position of a gene around the site to be mutated. But it contains mismatch of or the base to be mutated.
- The starting material is a single-stranded DN A (to be mutated) carried in an M_{13}, phage vector.
- On mixing this DNA with primer, the oligonucleotide hybridizes with the complementary sequences, except at the point of mismatched nucleotide.
- Hybridization (despite a single base mismatch) is possible by mixing at low temperature with excess of primer, and in the presence of high salt concentration

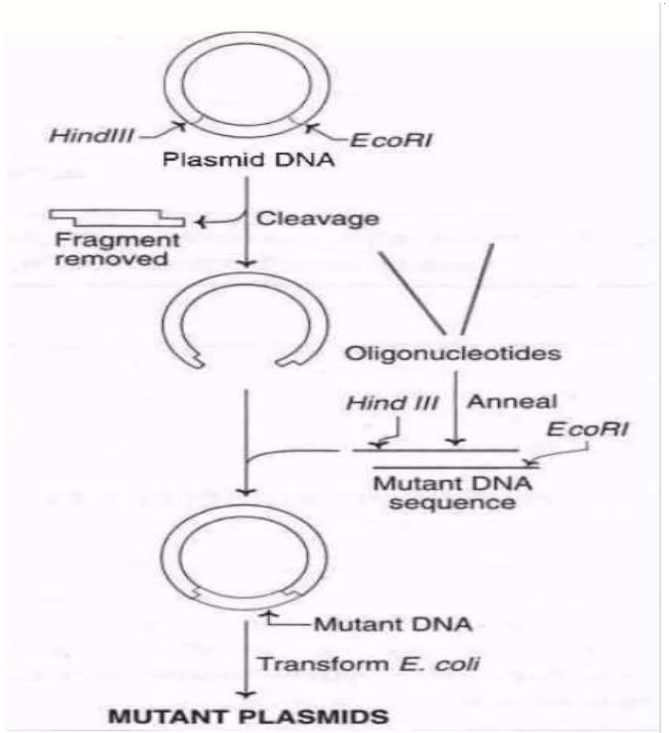


- The addition of 4-deoxyribonucleoside triphosphates and DNA polymerase(usually klenow fragment of *E.Coli* DNA polymerase) replication occur.
- The oligonucleotide primer is extended to form a complementary strand of the DNA.
- The ends of the newly synthesized DNA are sealed by the enzyme DNA ligase.
- The double-stranded DNA (i,e. M phage molecule) containing the mismatched introduced by nucleotide into *E .coli* transformation .
- The infected *E. Coli* cells produce M_{13} virus particles containing either the original wild type sequence or the mutant sequence.
- It is expected that half of the phage M_{13} particles should carry wild type sequence while the other half mutant sequence (since the DNA replicate semiconservatively).
- The double-stranded DNAs of M_{13} are isolated.
- Oligonucleotide –directed mutagenesis by using plasmid DNA (instead of M_{13}) is also in use

Localized Random Mutagenesis(Cassette Mutagenesis)

- CASETTEE MUTAGENESIS

- In casettee mutagenesis a, synthetic double stranded oligonucleotide (a small DNA fragment i.e., casettee) containing the requisite/desired mutant sequence is used.
- Casettee mutagenesis is possible if the fragment of the gene to be mutated lies between two restriction enzyme cleavage sites.
- This intervening sequence can be cut and replaced by the synthetic Oligonucleotide (with mutation).
- The plasmid DNA is cut with restriction enzymes (such as EcoR1 and Hind111).



Chemical Modification

- It is modification of enzyme or protein by modifying gene structure at a post-transational stage of the central dogma.
- Here the introduction of a new chemical group before translation results in the formation of modified enzymes.
- For example: PEG modification of enzyme L-asperginase i.e PEG-L-Asperginase formation becomes more effective than its native enzyme.
- L-asperginase has anti-tumour activity but toxic while modified form improves biostability and non-allergic.
- Here the gene sequence is isolated from L-asperginase enzyme and traced with PEG to give mutant gene thus gene is expressed to give the PEG-L-asperginase enzyme conjugate.

Application

- **Biomedicine:** Protein engineering can be used to create new drugs, vaccines, and diagnostics.
- **Agriculture:** Engineered proteins can be used to improve crop yields, protect crops from pests and diseases, and enhance plant growth.
- **Bioremediation:** Engineered proteins can be used to clean up environmental contaminants.
- **Industrial biotechnology:** Engineered proteins can be used in industrial processes, such as the production of biofuels and bioplastics.
- **Structural biology**: Protein engineering can be used to study the structure and function of proteins, which can provide insight into biological processes.
- **Materials science:** Engineered proteins can be used to create new materials with desirable properties, such as strength, elasticity, or biocompatibility.
- **Biosensors:** Engineered proteins can be used to detect a wide range of analytes, such as pathogens, toxins, or chemical pollutants.
- **Diagnostics:** Engineered proteins can be used to detect diseases or other health conditions in a quick and cost-effective manner.
- **Therapeutics:** Engineered proteins can be used to treat a variety of diseases, such as cancer, genetic disorders, and autoimmune diseases.