PROTEIN ENGINEERING

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INTRODUCTION

- Protein engineering can be defined as the modification of protein structure with recombinant DNA technology or chemical treatment to get a desirable function for better use in medicine, industry and agriculture.

OBJECTIVES OF PROTEIN ENGINEERING

- The objectives of protein engineering is as follows –

(a) to create a **superior enzyme** to catalyze the production of high value specific chemicals.

(b) to produce **enzyme in large quantities**.

(c) to produce **biological compounds**(include synthetic peptide, storage protein, and synthetic drugs) **superior to natural one**.

RETIONALE OF PROTEIN ENGINEERING

For industrial application an enzyme, should possess some characteristics in addition to those of enzymes in cells.

These characteristics are :-

- (1) enzyme should be robust with long life.
- (2) enzyme should be able to use the substrate supplied in the industry even it differs from that in the cell.
- (3) enzyme should be able to work under conditions, e.g. extreme of pH, temperature and concentration of the industry even if they differ from those in the cell.

- In view of above, the enzyme should be engineered to meet the altered needs. Therefore efforts have been made to alter the properties of enzymes.

- These are some character that one might have to change in a predictable manner in protein engineering or enzyme engineering to get the desired function :-

Character that one might have to change

- Kinetic properties of enzyme-turnover and Michaelis constant, K_m.
- Thermo stability and the optimum temperature for the enzyme.
- Stability and activity of enzyme in non-aqueous solvents.
- Substrate and reaction specificity.
- Cofactor requirements
- Optimum pH.

- Therefore for a particular class of enzymes, variation in nature may occur for each of the above properties, so that one may like to combine all the optimum properties to the most efficient form of the enzyme.

- For an e.g. glucose isomerases, which convert glucose into other isomers like fructose and are used to make high fructose corn syrup vital for soft drink industries.

Basic assumption for protein engineering

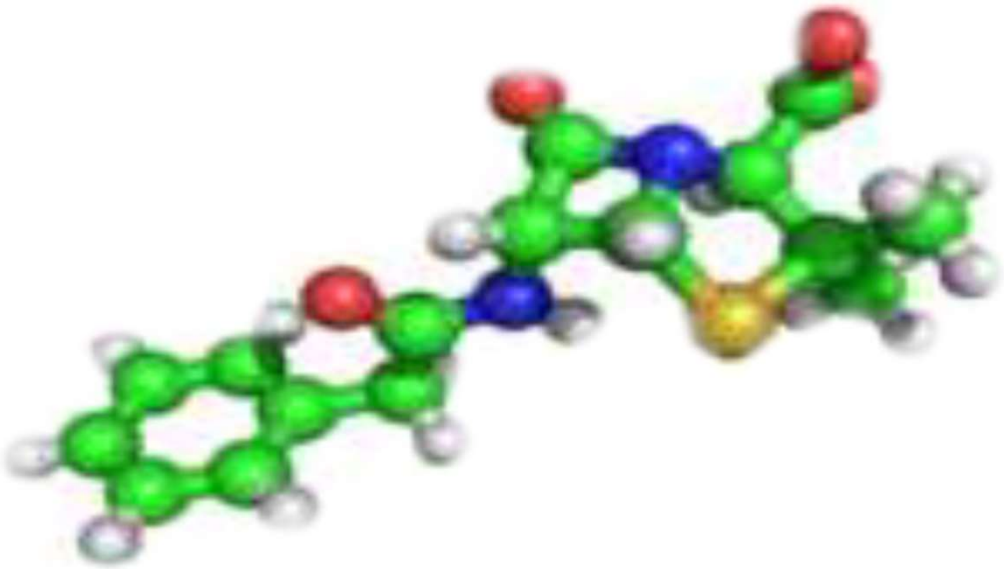
While doing protein engineering one should recognize the following properties of enzymes,

- Many amino acid substitution, deletions or additions lead to no changes in enzyme activity so that they are silent mutator.
- Protein have limited number of basic structures and only minor changes are superimposed on them leading to variation
- Similar patterns of chain folding and domain structure can arise from different amino acid sequences with little or no homology.

Steps involved in protein engineering

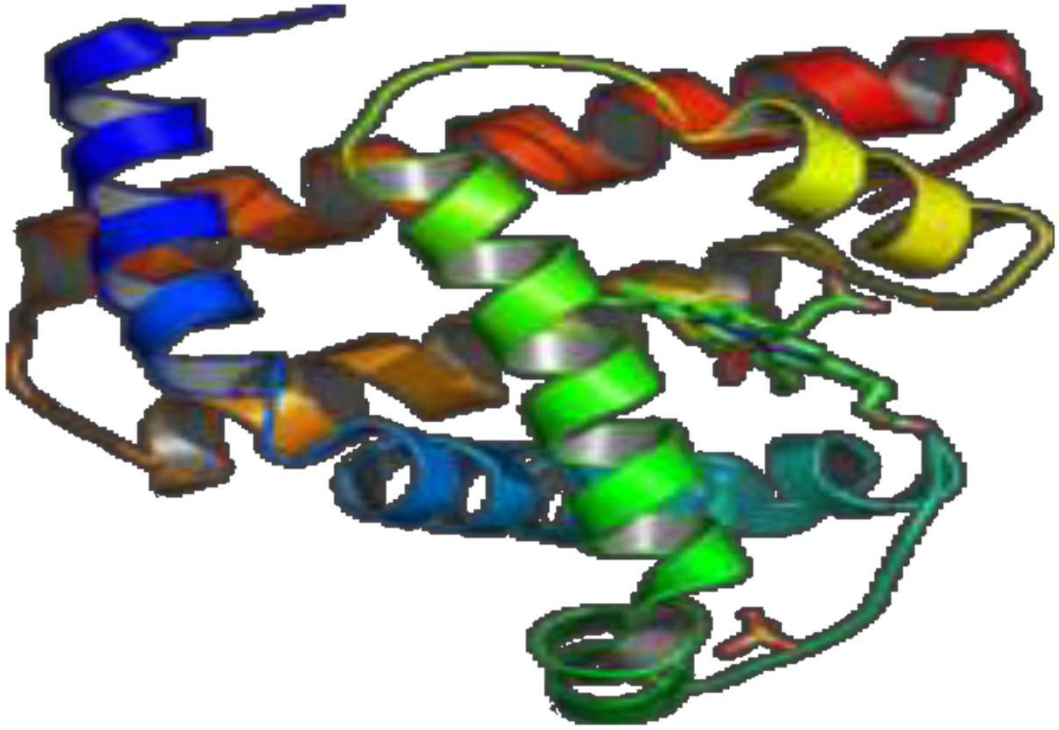
A study of three dimensional structure of protein :-

- A study of three dimensional structure is the preliminary steps of protein engineering.
- And a 3D structure of protein is produced from the data generated from X-ray crystallography and NMR process by protein modeling.



- The three-dimensional structure of penicillin, for which Dorothy Crowfoot Hodgkin was awarded the Nobel Prize in Chemistry in 1964.

- The green, white, red, yellow and blue spheres represent atoms of carbon, hydrogen, oxygen, sulphur and nitrogen, respectively.



- Ribbon diagram of the structure of myoglobin determined with the x-ray crystallography

Methods for protein engineering

- A variety of methods are used in protein engineering such as
- Mutagenesis, selection and recombinant.
- DNA technology.

Mutagenesis

- Mutagenesis and selection can be effectively utilized for improving a specific property of an enzyme.

E.g. for *E.coli* **anthranilate synthase** enzyme is normally sensitive to **tryptophan inhibition** due to feedback inhibition but an altered MTR2 mutation of *E.coli* was found to possess an altered form of enzyme anthranilate synthase that is insensitive to tryptophan inhibition.

And thus helping in the continuous synthesis of tryptophan without inhibition.

Gene Modification

The two process of gene modification are-

- (a) *In vitro* mutagenesis using synthetic oligonucleotides.

- (b) Synthesis of complete modified gene de novo.

(A) In vitro mutagenesis using synthetic oligonucleotides.

- Synthetic oligonucleotides is used for *In vitro* mutagenesis.

  In this method a small oligonucleotides primer containing the desired modification is first **synthesized**. It is then **hybridized** to the appropriate site and **cloned** then the rest is **replicated** using DNA polymerase enzyme, so that the rest remains unaltered.

- This approach is actually used to modify the active site of the tyrosyl-tRNA synthetase

(B) Synthesis of complete modified gene de novo.

- Complete gene in some cases have been chemically synthesized in the form of several oligomers (e.g. genes for insulin, somatostatin and interferon), that are ligated in correct order to produce a complete gene.

- The sequence of the synthetic gene can be designed in a modular fashion to get the desired function.

Chemical modification of enzymes

- The protein synthesized under the control of gene sequence in a cell undergo post transitional modification. This leads to stability, structural integrity, altered solubility and viscosity of individual proteins.

for e.g. Enzyme-PEG conjugates. An enzyme L-asparaginase has anti-tumour properties but is toxic with a life time of less then 18 hr. thus reducing its utility.

L-asparginase can be modified by polyethylene glycol derivatives to produce PEG-asparginase conjugates

RESULTS:

PEG-asparginase conjugates, which differ from the native enzyme in the following way

(i) It retains only 52% of the catalytic activity of the native.

(i) It become resistant to proteolytic degradation.

(iii) It doesn't cause allergy.

Achievements of protein engineering

- A number of proteins are known now where efforts have been made to know the effects of site specific mutagenesis involving substitution of one or more amino acids.

Ex:

- **Insulin-** it consist of A and B chains are linked by C-peptide of 35 amino acids. It was shown that a sequence of 6 amino acids for c-peptide was adequate for the linking function.

- **Cytochrome c –** A phenylalanine residue has been identified to be non-essential for electron transfer but is involved in determining the reduction potential of the protein.

- **Trypsin-** It could be redesigned to have altered substrate specificity.

THANK YOU

Happy to answer if you have any Question.....?