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Protein Engineering and its application in Plant Protection

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SUMMARY

Protein engineering is the process of developing useful or valuable proteins. It involves the understanding of protein folding and recognition for protein design. Protein engineering and directed evolution are effective tools for investigating protein sequence function connections. It is simpler to modify a protein using a gene. Protein engineering tools have no specific limitations. Any approach that can change the protein composition of an amino acid and result in a change in protein structure or function falls under the category of Protein engineering. It plays an important role in the plant protection area.

INTRODUCTION

The increasing demands for food, feed, fuel, and fibre are being challenged by global population growth and the simultaneous reduction of fertile agricultural land. Plant breeding in the traditional sense is insufficient and slow to improve crop qualities. Plant transformation technologies can introduce any gene with desired function into a crop of interest. A gene encoding a protein for a given trait from a given species will behave identically in the transgenic crop and confer the desired trait. To attain the end goal, the protein will have to be engineered or redesigned due to compromised expression, folding, and stability. Protein engineering is the process of changing the sequence of a protein by inserting, deleting, or substituting nucleotides in the encoding gene in order to create a modified protein that is more suited to a certain application or purpose than the original protein (Engqvist and Rabe, 2019). Protein engineering and directed evolution are powerful technologies for probing protein sequence function relationships. Protein engineering, which is based on genetic analysis, is the second type of genetic engineering. Genetic engineering can only produce the existing proteins in nature. Their forms and functions may be appropriate for the survival of a given species, but they fall short of meeting the growing demands of people's daily lives and industrial production. Protein engineering techniques applied to plants could help to solve these worldwide issues by increasing crop yield and quality.

Objectives

- To create superior enzymes to catalyze production of high value targeted product.
- To modified enzymes for large scale use in the industry.
- To produce biological compounds that are superior to natural ones.
- Enzyme should be able to use the substrate supplied in the industry even if it differs slightly from that in the cell.
- Proteins should be able to work under extreme conditions.

Strategies for Protein Engineering

Rational design: Based on the three-dimensional structure and the relationship between structure and function. The computer model allows predictions to be made, particularly in the realm of the effect of mutations on structure-based properties. The rDNA method of choice is commonly site-directed mutagenesis, in which one amino acid at a particular location is replaced with another.

Directed Evolution: Based on the process of natural evolution, no structural information required, no understanding of the mechanism required.

General Procedure:1. Generation of genetic diversity -Random mutagenesis
2. Identification of successful variants -Screening and selection

Methods for Sequence Diversification involves Error-prone PCR, Site saturation mutagenesis, DNA shuffling or chimeragenesis, Random mutagenesis using chemical agents, physical agents, or hypermutator strains. The purpose is to create a sequence library, which is a large collection of diverse sequences that includes potential solutions to the engineering goal, regardless of the approach utilised.

De Novo Protein Design: Main goal is to create any functional protein by simply specifying the sequence of amino acids that comprise it. A second major strategy in de novo design uses known protein structures as natural scaffolds to present a new property such as catalysis, inhibition, or metal binding sites (Rao, 2008).

Application of Protein Engineering in Plant Protection

- **Evolution of Bt Toxins:** Optimization of *Bacillus thuringiensis* toxin (Cry proteins) has been a focus of insect-specific pest control strategies. Optimization through: Truncation, Domain swapping, peptide addition, amino acid mutation (Deist *et al*., 2014). The use of both rational and directed evolution methods expands their applicability to a wider range of pests.
- **Engineering Plant Immune Effectors**: Intracellular immune receptors are an important part of the molecular mechanism that defends against pathogens. NBS- LRR -containing proteins bind to specific effectors found in pathogens and trigger a defense reaction. Random mutagenesis of these proteins can modify by error-prone PCR or site saturation mutagenesis and subsequent transformation employing *Argobacterium tumefaciens* (Segretin *et al*., 2014)
- **Crops Resistant to Herbicides:** The development of transgenic crops expressing proteins that make them herbicide resistant. Several attempts have been made to identify novel glyphosate-resistant mutants of ESP synthase by protein engineering. Engineering EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) to remain active in the presence of glyphosate or inserting genes encoding enzymes that break down glyphosate have been used to create glyphosate-tolerant transgenic plants. (Tian *et al.*, 2013).

CONCLUSION

Protein engineering and directed evolution are powerful technologies in biotechnology. These technologies have only been applied to a limited set of plant traits. Protein engineering mainly involves two general strategies, 'rational' protein design and directed evolution. Protein engineering involve modification of protein genetically or chemically. It plays an important role in the plant protection aspect.

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