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The role of protein engineering in the design and production of recombinant proteins

Abstract

Proteins possess many structural and functional characteristics that these features are not seen in any other biomolecules. So many biologists have been persuaded that using protein engineering design and build their desired proteins. These engineered proteins can act as new molecular tools for scientific, medical, industrial, etc. applications, so they can satisfy many human needs that are not met by natural proteins. Protein engineering based on calculations Strategy produces and screens the protein sequences with "in silico" (i.e. before its synthesis in the laboratory). Structure-based protein engineering calculations similarly use the calculations to discover the protein sequences. However, calculation-based protein engineering also emphasizes the new and useful protein engineering and investigating the relationship between structure and function of them. This study investigates the protein engineering tools in producing the recombinant and engineered proteins. For this purpose, bioinformatics studies, how to predict the second and third buildings based on homology and adaptive modeling, predicting protein performance for the production of recombinant proteins are investigated.

Keywords: protein engineering, recombinant protein, emphasizes, in silico, biomolecules

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Introduction

Due to the versatility of microbial recombinant proteins, practical features and easy production, they are considered an important group of products with biotechnology value.[1–7] This group of proteins, according to their careful design, possesses specific characteristics that are very attractive for various applications.[5] In the design and production of recombinant proteins, we must take care that between the complexity of a specific protein and complexity and capabilities of an expression system there exist a direct relationship so that single protein subunits are easily capable of producing in bacterial hosts, while proteins that require mammalian proper glycosylation or the presence of multiple disulfide bonds are forced to express in higher eukaryotic host.[6]

Given the complexity of engineering proteins they need a combination of computational and experimental approaches. ¬¬protein engineering strategies based on the variant changes identify the desired protein from among a large number of mutant variants. Therefore their success depends on a number of mutations required to apply appropriate methods for studying these mutations. On the other hand, protein engineering strategies based on calculations, produce and screen the protein sequences "in silico", (i.e. before its synthesis in the laboratory). Structure-based protein engineering calculations similarly uses the calculations to discover the desired protein sequences. However, calculation-based protein engineering also emphasize the new and useful protein engineering and investigate the relationship between structure and function of them.[8–12] Random mutant, recombination, and diversification directed at the three main groups are methods used for building libraries in protein engineering.[13]

Protein engineering purposes, especially in enzymes, is enlarging the active position, changes activity (change specific activity, change the characteristics of the substrate), sustainability (change thermal stability, protease stability and oxidation stability) and resistance to surfactants and detergents.[14–16] Schmidt and colleagues also used random mutation by error-prone, created a set of mutations for target enzyme to change anantio selectioiti.[13]

Mesophiltipic enzyme from B. subtilis LipA with the purpose of directed mutagenesis was put in place, one of the nine created mutations substantially increased the melting point of 15 degrees and 20 degrees in optimum temperature for mutant lipase activity compared with wild-type.[1] In 2009 the Factory et al. used Site-directed Mutagenesis method in Bacillus lipase enzyme termokatlonatous to reduce the space interference in active enzyme site for better access to the substrate, they replaced amino acid phenylalanine 181 and 182 with Alanine which leads to an increase in the enzymatic activity.[9] Protein engineering describes the process of altering the structure of an existing protein to improve its properties. It is an important technology that increases our basic understanding of how enzymes function and have evolved, and it is the key method of improving enzyme properties for applications in pharmaceuticals, green chemistry and biofuels. In the following sections, tools and databases in protein engineering and genetic engineering which are used in the design and production of recombinant proteins are discussed.

Protein sequence alignment

We can find similar protein sequence alignment from this NCBI website (http://www.ncbi.nlm.nih.gov/). According to this similarity finding, we can find the most similar protein to the expected protein in terms of sequence and structure. We can also predict the protected sequence, enzyme family, amino acids in the active site, oxyanion cavity etc. in target protein through BLAST in this database. To predict the presence of Disulfide bond, we can use DISULFIND datacenter (http://disulfind.dsi.unifi.it/). Also to determine the presence of signal peptide we can use Signal P 4.1 (http://www.cbs.dtu.dk/services/SignalP/). For a signal peptide, D must score higher than 0.45, as

(Figure 1) predicted score for protein (lipase) equal to 116/0. So in this forecast does not predict a signal peptide.

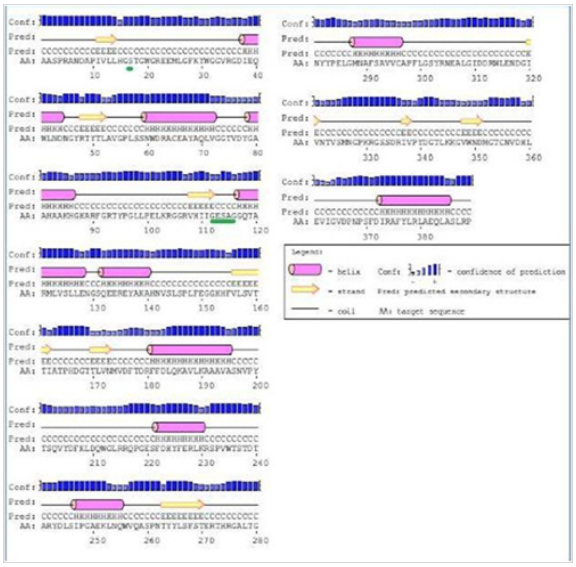

Figure 1 Predicting Signal Peptide.

Choosing the expression host

To select the host for protein expression we can use GenScript (http://www.genscript.com/). In this center, codon sequence of the desired protein is compared with codon usage of expression microorganisms such as yeast and *E. coli*. As the number CAI (Codon Adaptation Index) is closer to number 1, the expressed desired protein is higher.

Software study of protein secondary structure

To study the effect of mutations on recombinant protein secondary structure, the bioinformatics methods were used. For this purpose, the protein secondary structure prediction software is used in PSSPRED and YASPIN servers (3). The results of recombinant protein secondary structure prediction based on the scores of the natural amino acid changes resulting from mutations shows recombinant protein secondary structure. In this method the status of each amino acid is predicted according to its place in the type of secondary structure and the certainty of this prediction is displayed with numbers from zero to nine. (Figure 2) shows an example of the second structure of a recombinant protein.

Software study of protein tertiary structure

In determining the tertiary structure of recombinant proteins, first to determine the pattern, blasts against pdb from phyre server is done which at this website, the structure with the greatest homology is selected as a template. Then, the recombinant protein structure and the pattern is predicted and optimized using modeler, Easy MODELLER and PYTHON software and is displayed using the VMD software and thus the third structure is made.[15]

Assessment of ppredicted recombinant protein structures

Study of normal and mutant lipase structures using adaptive method

To study the three-dimensional structure of recombinant proteins, first, the structure of these proteins has been predicted and optimized using Easy MODELLER software. Then the recombinant protein is matched on a natural protein. The RMSD (Root Mean Squar deviation) between atoms in two molecules is measured and calculated according to Å. This number is between zero and one, and as the amount of RMSD is closer to zero, it indicates that the mutation has not changed the overall structure of recombinant protein and these proteins are matched on each other.[8]

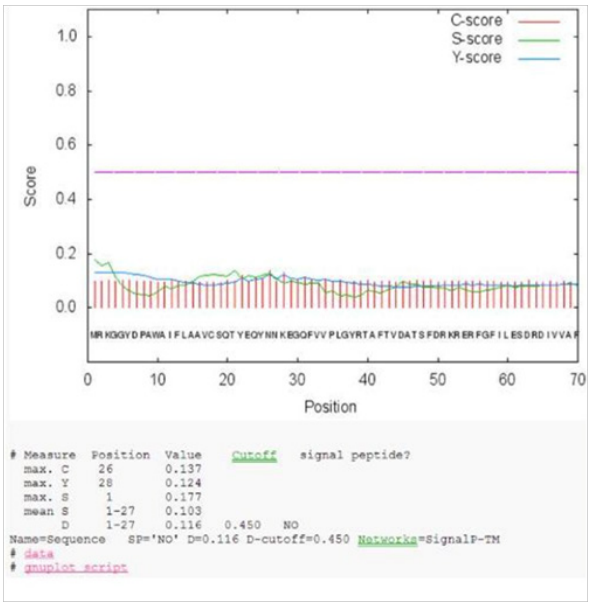

Figure 2 Recombinant Protein Secondary Structure Prediction.

Evaluation of the structure using ramachandran diagram

Second structures of proteins are created by Sai and Phi angles and Ramachandran diagram shows the authorized status of each angle for protein structures. In fact, this chart is a way to visualize the angle φ against ψ backbone of amino acid residues in the protein structure.[4]

Evaluation of protein structure through energy profile

Server proSA (https://prosa.services.came.sbg.ac.at/prosa.php) determines the quality of predicted structure based on Z-Score points and also determines the energy profile. As Absolute magnitude of Z-Score is closer to 10, the structure has a higher quality and the more negative energy profile shows a higher structure quality.[2]

Function prediction of normal and mutant Lipase proteins

For predicting the function of recombinant protein, the interaction of various ligands with the recombinant and natural protein is investigated using MVD (Molegro Virtual Docker) software.[11] As a result of this ligand-protein connection by providing the MolDock score, interaction is estimated. MolDock, Escore is defined by the following energy expressions:

Escore = Einter + Eintra. In this regard Einter is the protein-ligand interaction energy and Eintra shows the ligand internal energy. The internal energy of ligand here is the same for each ligand and the effective energy in this equation depends on the energy of ligand-protein interactions and as this energy is less (more negative) the stability of the substrate at the active site is done better and substrate proteins are more stable. In studying the recombinant protein interactions with various ligands, it reveals that according to predictions done,

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how much is the required energy for recombinant and natural protein. Thus, we can predict the recombinant proteins function comparing to natural proteins (Especially in the case of enzymes).

Conclusion

Today, knowledge of enzymology has created profound changes in biotechnology industries. Until the 60s, the income of industrial enzymes was only a few thousand dollars a year, but by the growth of this industry in recent years, this income has increased. Today, most of enzymes are prepared by fermentation of bio-based materials. Protein engineering techniques are highly efficient in producing industrial enzymes.

Enzymes derived from microbial sources using protein engineering and molecular techniques are very useful because they can be produced at low cost and they show improved stability. So the production of recombinant proteins should be economically feasible and gene technology must be able to provide tools to compete with traditional sources, and produce technical enzymes and food additives. Thus, the use of molecular techniques and a tool called protein engineering lead to production of efficient systems and inexpensive components for cultivation in various processes.

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Conflicts of interest

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