REVIEW

Pharmaceutical and industrial protein engineering: Where we are?

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Abstract: The huge amount of information, the big number of scientists and their efforts, labs, man/hrs, fund, companies all and others factors build the success of the amazing new branch of genetic engineering the *'Protein Engineering*' (PE). It concerns with the modification of protein structure/function(s) or building protein from scratch. The engineered proteins usually have new criteria(s). Engineering proteins can be mediated on the level of genes or proteins. PE fined its way in different important sectors including industrial, pharmaceutical and medicinal ones. Aspects about PE and its applications will be discussed with this review. The concept, tools, and the industrial applications of the protein, engineered proteins and PE will be under focus. In order to get up to date Knowledge about the applications of PE in basic protein and molecular biology, several examples are discussed. PE can play a significant role in different industrial and pharmaceutical sectors if used wisely and selectively.

Keywords: Protein engineering; applications; rational design; directed evolution.

Protein

Proteins are macromolecules, which participate in every process of different cells. Protein name derived from the Greek word *prðtos,* meaning "first" or "foremost". The Swedish chemist Jöns Jakob Berzelius was the first to describe protein as a term in 1838. James B. Sumner (1926) showed that urease is a protein. Changing protein composition is a natural phenomenon, e.g., Sickle cell anaemia and Cystic fibrosis (Harris, 1992; Platt and Falcone, 1988). Post-translation modification, which can happen either before the protein is used in the cell, or as a part of control mechanisms is a natural mechanism for modifying protein structure (Caraglia1 *et al.,* 2004).

Proteins work together to achieve a particular function, and they often associate to form stable complexes (Anthea, 1993). Insulin was the first protein to be sequenced, by Frederick Sanger, who won the Nobel Prize for this achievement in 1958. He has succeeded in solving the insulin sequence using protein amino acids sequencing rather than using the insulin related genes. It was a complicated process especially in the presence of disulfide bonds between A and B fragments. However, Sanger and Thampson (1953a,b) have identified the importance of the insulin 3D structure. The first three-dimensional (3D) structures for hemoglobin and myoglobin have been resolved by Muirhead and Perutz (1963) and Kendrew *et al.,* (1958 a,b) respectively. The 3D structures of both proteins were determined by x-ray diffraction analysis. On the basis of their pioneering work, they were both awarded Nobel Prize in Chemistry in 1962.

Rapid advances in the related fields (including DNA isolation and purifications, cloning, sub-cloning, sequencing, site-directed mutagenesis, gene synthesis, new expression systems in prokaryotic and eukaryotic cells) have provided the molecular biologist additional tools for modifying existing proteins to improve their catalytic activities, stability, and selectivity. Different results and information about protein structure/function(s) have been used by different researcher to improve their design. Protein structure/function(s) knowledge has been found its applications in different fields including food industry, enzymes, pharmaceutical products, protein folding applications, targeted drugs, nanomedicine, hormones (insulin), growth factors, protein crystallization, antibody-antigen docking etc. (Goodenough 1995; Runbingh 1997; Arulmuthu *et al.,* 2009; Crisman and Randolph 2009; Debbage, 2009; Huang *et al.,* 2009; Sivasubramanian *et al.,* 2009; Kang and Jang 2009).

Protein is highly specific and could produce complicated processes like DNA and RNA polymerase, where the DNA polymerase reaction is catalyzed by two-metal ion mechanism in the 3'-exonuclease reaction. In contrast RNA polymerase do more complicated function while it work in one ribonucleic acid strand only. They are able to initiate RNA synthesis without requiring a primer oligonucleotide exhibit an abortive initiation phase and

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"Any change in the gene/protein lead to a new structure/function(s) is in the frame of PE" *The author*

they are target of a host regulatory proteins (activators, inhibitors, terminators and anti-terminators) that modulate gene expression (von-Hippel *et al.,* 1984; Erie *et al.,* 1992; Ikeda *et al.,* 1986; Brautigam and Steitz. *et al.,* 1988; Freemont *et al.,* 1988; Beese and Steitz, 1991).

Usage and importance

There are many concepts behind improving the quality of protein preparation for use in particular applications. In commercial applications, proteins can be less pure or completely crude; however, they must be free from any contamination in case of medicinal applications. Pharmaceutical and medically used proteins should be highly pure, active, correctly folded and biocompatible (Degim and Çelebi, 2007; Woods and Hamuro 2001; Nishijima 2005; Standley *et al.,* 2008). Regarding the activity and stability, the protein must match perfectly the reason of its usage. Although, the use of PE in industrial applications has been increased significantly, screening of wild types organisms containing new proteins with particular function(s) should not be stopped (Satyanarayana *et al.,* 2005). Organisms from extreme environments are becoming an important source of new backbones for engineering proteins with significantly different properties and flourished the protein databases with amazing structures (van den Burg, 2003). Successful engineered proteins in general require proper combination of their properties. For example, protease used in Powder-detergent would require stability against certain protein stains. Properties, such as stability, high activity (in the case of enzymes) and the ability to fold and interact correctly with surfaces are necessary for a variety of industrially important proteins (Goodenough 1995; Runbingh 1997; Arulmuthu *et al.,* 2009; Crisman and Randolph 2009). PE should be applied to solve complicated problems and introduce unexplored new properties (Chen *et al.,* 2002; Fowler *et al.,* 2002; Kan 2002; O'Maille *et al.,* 2002; Best *et al.,* 2003; Kiss *et al.,* 2003; Nielsen *et al.,* 2004b; O'Maille *et al.,* 2004; Sueda *et al.,* 2004; Tandang *et al.,* 2005; Bai *et al.,* 2007; Lin *et al.,* 2007; Alahuhta *et al.,* 2008; Evdokimov *et al.,* 2008).

Manipulation of protein through gene

Manipulation and modification of proteins chemically or through its constituent of amino acids is a complicated process, however modification of proteins through genes has been proved as an easier and more efficient approach. The basic techniques of genetic engineering are altering the responsible genes and generate the proteins with novel activities or properties. Such manipulations have frequently been used to discover structure/function(s) relationships, as well as to alter the activity, stability and properties of proteins (Gan *et al.,* 2002; Milik *et al.,* 2003).

Rational design-the logical approach

Rational design plays a crucial role in PE Phenomena and manipulate different complicated processes such as regenerative medicine, tissue engineering, protein delivery system, cell adhesion interaction modulation, signal transduction, understanding the protein-protein interface and analysis the protein 3D (Moss *et al.,* 2009; Costa *et al.,* 2002; Liu *et al.,* 2007; Holmwood and Schindler 2009). Rational design has been improved by modification in the site directed mutagenesis methods, protein 2D and 3D, structure modelling, protein-protein interaction and their related software (Chie *et al*. 2004; Chie *et al*. 2005; Boeris *et al*. 2009; Kurgan 2008; Ludwig *et al.,* 2003; Shen *et al*. 2007; Sugimoto *et al*., 2004; Yan *et al*. 2008).

The concept of protein 3D modeling is based on relating a structure to the possible expected function(s) of amino acids residues (homology modelling) and replacing them with other functions (molecular dynamics) in order to change their properties (Visegràdy *et al.,* 2001). Rational design requires some knowledge about the basic protein principle, genetic engineering tools and good skills in mathematics and algorithms and computing different software have been introduced to facilitate and reduce the steps incorporated in the PE (Du *et al*. 2009; Quine *et al.,* 2004).

Engineering protein is a sensitive and systematic process that can get a breakdown if any mistake is committed. In case of unwanted addition or loss of one or more base pair during the sequencing process or stepwise analysis will lead to DNA frame shift, causing a change in its amino acids sequences. Important role of protein modelling was represented in designing a protein 3D structure from other protein x-ray-structures (Chappell *et al.,* 2002; Gaudier *et al.,* 2002; Ozbek *et al.,* 2002; Rhem *et al.,* 2002; Vannini *et al.,* 2002; Woo *et al.,* 2002; Akarsu *et al.,* 2003; Calderone *et al.,* 2003; Feinberg *et al.,* 2003; Yun *et al.,* 2003; Ifuku *et al.,* 2004; Parker *et al.,* 2004; Timsit *et al.,* 2006; Molina *et al.,* 2009). Building a complete hypothetical model for protein did not crystallized yet is available by using the available protein structure database (Christendat *et al.,* 2002; Choi *et al*. 2009; Han *et al.,* 2005; Hattori *et al.,* 2005; Holmes *et al.,* 2006; Han *et al.,* 2008; Jang *et al.,* 2009).

Different computational algorithms have been introduced to identify the amino acid sequences that have low energies for target structures. The sequence-conformation space under investigation is usually large. Software for proteins modeling has been established with different ability to detect the presence of un-natural molecules (Anfinsen, 1972; Briki *et al.,* 2002; Dana *et al.,* 2006; Chen *et al.,* 2007; Cavasotto *et al.,* 2008; Bordoli *et al.,* 2009; Chen *et al.,* 2009; Chen and Shi 2009).

Some of the software is able to display and manipulate the protein 3D structures (defined by X-ray analysis).

Continues development of different software, instruments, x-ray machines, synchrotrons etc., together with advancement in the field of molecular biology and genetic engineering brought tremendous progress in the fields of PE (Fieulaine *et al.,* 2001; Thore *et al.,* 2003; Ball, 2008; Herrmann *et al.,* 2002a; Herrmann *et al.,* 2002b; Calderone 2004; Yao *et al.,* 2006; Fukunishi and Nakamura 2008; Abendroth *et al.,*; Procopiou *et al.,* 2004; Sugimoto *et al*. 2004; Yu *et al.,* 2004; Nicolini and Pechkova 2006; Pechkova and Nicolini 2006; Yu *et al.,* 2009).

Directed evolution

Directed evolution is an approach concerning with changes in the existing genetic material to modify its structure/function(s) (Yuen and Liu 2007). Natural evolution occurs under different pressures conditions but with capacity to save the organism from the newly activated pressure. Directed evolution has been developed using controlled or designed selection to introduce new selected function(s). It can be obtained through the design of successful selection protocol for the newly expected mutants.

The directed evolution is widely used to solve different industrial problems and to improve protein properties. It has also been cited in terms of molecular evolution, sexual PCR and *in vitro/vivo* evolution (Zhao *et al.,* 1997). It is the technique of preparing protein variants by recombining gene fragments *in vitro*, using PCR (Ko and Ma, 2005). After gene modification the new expressed protein can be subjected to another cycle(s) of modification (Crameri *et al.,* 1998).

***de novo* design of proteins [protein from scratch]**

Enhancement of knowledge about different protein structure/functions encourage scientist to design proteins *de novo* (Iwata *et al.,* 2001; Dai *et al.,* 2002; Matsuura and Pluckthun 2004; Khodagholi *et al*. 2008; Klepeis *et al.,* 2005; Takekiyo *et al.,* 2007; Zou *et al.,* 2007; Wang *et al.,* 2008; Shiga *et al.,* 2009). They used information about the molecular recognition, conformational preferences, and structure analyses of native proteins together with latest software for data analysis and manipulations. The design of new proteins is based on the available information, especially those about protein 3D structure. Any kind of knowledge about 2D and 3D protein structure will be of great important (Bryson *et al.,* 1998). One example about the newly designed protein is α-helical peptides that catalyze helical peptide ligation, and was first reported by Ghadiri and co-workers (Severin *et al.,* 1997). The positioning of the peptide substrate for catalysis is due to hydrophobic and electrostatic interactions between the two helices (Scheraga, 1985). Peptide ligation depends on the reaction between an N-terminal cysteine and a C-terminal thioester to form an amide bond between two peptide entities. Although, the design of new larger protein is restricted to redesigning of the existing ones, building new proteins is successfully achieved especially for their active sites. The re-design of the hydrophobic core has been performed on number of proteins, including the four-helix bundle ROP (Munson *et al.,* 1994) and ubiquitin (Munson *et al.,* 1994; Lazar *et al.,* 1997).

Chemical synthesis

Peptide synthesis can be produced chemically using organic synthesis techniques (Guzmán *et al.,* 2006; Bang *et al.,* 2005). Chemical ligation is used to produce peptides in high yield. The use of different strategies for chemical synthesis allows the researcher to introduce un-natural amino acids into the protein polypeptide chains, such as attachment of fluorescent probes to amino acid side chains. However, these methods can only be used on small scales (such as in the laboratory) but not on commercial scale (Bray *et al.,* 2003). Chemical synthesis has been determined inefficient for polypeptides having more longer than 300 amino acids (Mirzaei *et al.,* 2008). The synthesized proteins may not readily assume their native tertiary structure (Finkelstein and Galzitskaya *et al.,* 2004**)**. Most chemical synthesis methods proceed from C-terminus to N-terminus, which is almost opposite to the biological reaction (Ostermeier 1999; Schmidt *et al.,* 2001). However, chemical synthesis of protein is highly important, especially on the peptide level, while manipulating protein through gene is easier (in most cases). Meanwhile, manipulating short peptide through chemical synthesis is of great importance especially in pharmaceutical applications (Qabar *et al.,* 1996).

Mutagenesis

Change of amino acid(s) in a particular protein could happen naturally and lead in many cases to sever human diseases (Kao *et al.,* 2000). Chemical mutagenesis is an old method used for long time in strain improvement but in most cases it did not deliver where the mutation(s) has been happened (Chiang, 2004). The mutated DNA sequence using chemical mutagenesis is usually unknown (Chiang, 2004). Mutant induced chemically or by radiation can happen in any gene(s) (Ermakova-Gerdes *et al.,* 1996**)**. It is the role of the selection process to select the correct mutant (Michel *et al.,* 2000**)**. Moreover, it is important here to state that the mutagenesis should be wisely used. First priority will be for isolating of different kinds of proteins from nature. However, in certain cases particular protein needs to be engineered (Baum *et al.,* 2004). It is important to direct the power of PE correctly and the screening of the existing natural protein(s) should not be stopped and should continue on priority basis.

Site directed mutagenesis

"The first step from the filed of genetic engineering to protein engineering"

The use of PE started in 1978 when a site directed

mutagenesis was introduced in the laboratory of Michael Smith (Hutchison *et al.,* 1978). Michael Smith and his co-workers were the first to introduce synthetic oligonucleotides mediate mutations (Hutchison *et al.,* 1978). The first modification of an enzyme, a tyrosyl-transfer RNA synthetase, using these tools was performed in 1982 (Winter *et al.,* 1982). Through site-directed mutagenesis a cysteine was replaced by a serine altering the protein's substrate binding characteristics. His revolutionary work within this field earned Michael Smith the Nobel Prize in 1993. Many alterations in a particular protein can be generated by making amino acid replacements at specific site in the polypeptide backbone. Each protein is unique in its amino acids composition. At any position in the sequence, an amino acid can be replaced by another using site directed mutagenesis method (Flaman *et al.,* 2001; Bennett *et al.,* 2003; Ho *et al.,* 2005; Kasrayan *et al.,* 2007; Choi *et al.,* 2009; Edelheit *et al.,* 2009). As an example, Pancreatic ribonuclease A is an enzyme comprising 124 amino acids, which cleaves the covalent bonds by the joining of ribonucleic acids (RNA). If at position 119 in the sequence the naturally occurring histidine is replaced by an alanine, this mutant protein is expected to have little or no biological activity, because histidine 119 is important for that activity. Other mutations have very little effect on their proteins. This is particularly true when the amino acid is substituted by other closely related amino acids and when the amino acid is not conserved in the same protein found in other organisms (Branningan and Wilkinson 2002; Lippincott-Schwartz and Patterson 2003; Wang and Schultz 2002). Another example about site directed mutagenesis will be discussed in the following sections of the review.

Deletion and Insertion mutants

Different amino acid can be deleted from the sequence, either separately or in groups (Chen *et al.,* 2003). These processes are referred to as deletion mutants. Deletion mutation is usually used to map important region in a particular protein. Or, to reduce the protein size, by deleting apparently unnecessary part of a specific function. This usually results in missing one or more functions or properties of the wild type protein. It has been proved useful in creating smaller proteins that match perfectly the demand of different applications and is used to understand the importance of each part of the protein (Tratschin *et al*., 1984).

Whenever the deleted part impaired the protein function, that mean the protein active sites have been either partially or completely deleted (Liu *et al.,* 2000). Deletion mutation has been used to get rid of certain properties; in contrast, insertions mutation deal with inserting different amino acid relate base pairs within the gene sequence (Vorberg *et al.,* 2001; Fernando *et al.,* 2002; Ermakova *et al.,* 2003; Nielsen *et al.,* 2004a; Yang *et al.,* 2005; Brown and Maloy 2006; King *et al.,* 2006; Ellrott *et al.,* 2007; Jiang and Blouin 2007; Tang *et al*., 2007; Thaa *et al.,* 2008).

Hybrid/fusion proteins

Protein sequences can be joined or fused by another protein. The resulting protein is then called a hybrid, fusion, or chimeric, which generally has characteristics of the joint proteins. Protein fusions have been extensively used to study an interaction between two or more proteins (Kang and Jang 2009; Rehm *et al.,* 2002; Ludwig *et al.,* 2003).

Random mutagenesis

The probability that need to change particular protein is very high. Proteins, which are compose of 20 different amino acids, need significant number of site directed mutagenesis steps to alter its structure/functions. Random mutagenesis become a best choice when there is a shortage of existing information about protein structural/function(s) and the role of active residues (Amara *et al.,* 2001, 2002; Amara, 2003). Random mutagenesis is applied to a gene coded for a protein. The process passes through a selection protocol(s) that enable to select newly mutated genes by detecting a difference in the expressed protein behaviours after the mutagenesis steps. Genes could be a subject of more than one cycle of mutagenesis (Greener *et al.,* 1997). Random mutagenesis usually seems to be time consuming process, but alternative methods will be impossible if there is insufficient knowledge about protein structure/functions. Random mutagenesis mimics what could happen naturally that satisfies all the criteria leading to mutate certain gene; even randomly but with more force and directed strategy. Random mutagenesis can be used either *in vivo* using mutator strains or *in vitro* using different kinds of PCR random gene modification protocols. As an example a detailed *in vivo* random mutagenesis protocol for mutated $phaC_{Ap}$ synthase gene has been described by Amara *et al.,* 2002. The protocol has given a direction to select mutates of $PhaC_{Ap}$ synthase with enhanced activities, change the substrate specificity and give polymers with different monomeric composition. The *in vivo* random mutagenesis is based on mutating strain *E. coli* XL1 Red (Stratagene©) that is impaired in three of the DNA repairing mechanism pathways (Glickman and Radman, 1980).

Interestingly the changed amino acids were not in the active site, which indicate a clear role of the structure/function(s) relationship over the enzyme active sites as reported by Amara *et al*., (2001, 2002). Taguchi *et al*., (2001, 2002) used another strategy based on *in vitro* mutagenesis for $phaC_{Re}$ using PCR. It is important to highlight that a successful selection method should be used to efficiently recover the mutated gene from the wild type gene.

DNA shuffling

The DNA shuffling technique, introduced by Stemmer (1994), sets the directed mutagenesis approach apart from the earlier random mutagenesis, which enable to combine different DNA fragment from different proteins variants randomly using PCR. The screening efforts of Crameri *et al.,* (1998) have successfully applied this approach to improve the whole cell fluorescence by green fluorescent protein, which is widely used as a reporter for gene expression and regulation (Stemmer 1994; Crameri *et al.,* 1998).

You and Arnold (1994) in their study show a 471-fold increase in the activity of subtilisin E in 60% aqueous dimethylformamide. The engineered enzyme can function in organic solvents, which is different from its natural environments (You and Arnold, 1994).

Tailoring fundamental properties

Stability, activity, and surface properties are the most important criteria of technical enzymes. Isolation of enzymes with extra properties like extremophilic enzymes can be used to satisfy some of the PE demands that reduce the amount of engineered protein (Schäfer *et al.,* 2005; Leuschner and Antranikan 1995; Gomes *et al.,* 2004).

PE Applications

It increases an understanding about the enzyme properties, such as stability, activity, surface properties, purification and introduction of new applications.

Technical enzymes

In contrast to other fields of science, research which attracts the attention of industrial sectors is concerned with both cost and profit.

Technical enzymes are one of the major biotechnological industrial products, which had a market share of about 1 billion USD in 1999 (Schäfer *et al.,* 2005). Part of these products is the thermostable enzymes, which are well consumed in different industrial processes and constitute more than 65% of the worldwide market (Leuschner and Antranikan, 1995; Rao *et al.,* 1998). Enzymes have been used in many important industrial products. Their applications can be made in paper industry, detergent, drugs, degradation of different wastes, textile, food, pharmaceutical, leather, degumming of silk goods, manufactureing of liquid glue, cosmetics, meat tenderization, cheese production, growth promoters etc (Leuschner and Antranikan, 1995; Rao *et al.,* 1998; Gomes and Steiner, 2004; Cowan, 1996). Proteases have been used in many industrial processes including detergent, wool quality improvement, meat tenderization, leather, etc, (Amara and Serour 2008; Gupta *et al.,* 2002a; Gupta *et al.,* 2002b; Poza *et al.,* 2007; Tang *et al.,* 2004; Thangam and Rajkumar 2002; Valer, 1975). Ideally, the proteases used in detergent formulations should have high activity and stability within a broad range of pH and temperatures, and should be compatible with various detergent components along with oxidizing and sequestering agents (Horikoshi 1999; Ito *et al.,* 1998). PE has been used to improve the stability of BPN' from *Bacillus amyfoliquefaciens* in the chelating environment of the detergent by deleting strong calcium-binding site (residues 75-83) and re-stabilizing the enzyme through interactions without involving the metal-ion binding. Stability increase of more than 1000-fold in 10 mM EDTA have been reported for this protease (Strausberg *et al.,* 1995).

The surface properties of BPN' have also been engineered. Variants of mutates produce negative charges in the active site region of the molecule adsorbed less strongly (Broda *et al.,* 1996) and gave better laundry performance (Brode *et al.,* 1966; Rubingh, 1996a, b). Cold-adapted maturation of thermophilic WF146 protease by mimicking the propeptide binding interactions of *Psychrophilic subtilisin* S41 has been designed by Yang *et al* (2008).

Pharmaceutical applications

By rough estimate, about 350 biotechnology drugs currently undergo development. These include vaccines, gene therapy, antisense technology, and antibodies derived from 'humanised' transgenic mice (Whittingham *et al.,* 1997; Muller *et al.,* 2004).

PE has been used to produce therapeutic pharmaceutical proteins with improved properties such as increased solubility and stability.

Major protein based drugs applications problem

Different proteins which show activity *in vitro* and have a promising role in medicinal applications have been filed. The reason is that they are primary molecules with suboptimal affinity and poor half-life *in vivo*, which lead to poor efficacy (Moore *et al.,* 1993). In other cases, many of the original protein drug molecules are nonhuman that caused immune responses against the drug itself. Affinity, half-life, and dosing regime are all inter-related and play their role in determining the clinical efficacy and financial viability of protein-based drugs. This enhanced understanding about the issues affecting a success in drug development has been augmented by increased capabilities in PE and selection/screening technologies. These technologies have been used to improve the effectiveness of a number of proteins used as drug (Ki *et al.,* 2003).

Reducing the immunogenicity of protein drug molecules

Different non-human proteins, which appear *in vitro* high activity, are bonded by the immune system. This lead to focusing on using protein from human sources/or

humanized protein (Conner *et al.,* 1998; Shen *et al.,* 2006).

For example Pulmozyme (Genentech) is human DNAse derived drugs used in managing cystic fibrosis and bovine pancreatic DNAse I (Shak *et al.,* 1990). The immunoginicity of mouse antibodies in human protein was one of the major problems of the early monoclonal antibodies. Chimaeric antibodies by fusing mouse variable domains to human constant domains improve the body acceptance. This chimaeric retain binding specificity and reduce the amount of mouse sequence in their backbone.

In 1998, Remicade (Centocor), a TNFa-neutralising chimaeric monoclonal antibody, was approved for use in treating Crohn's disease and rheumatoid arthritis (Breedveld, 2000). A reduction in monoclonal antibody immunogenicity has taken a stage further by complementarily-determining region (CDR) grafting, where the CDRs of mouse antibodies were grafted onto human frameworks to further reduce the proportion of mouse sequences in the drug while retaining its binding specificity (Jones, 1986).

Examples

Insulin

Insulin (lispro and aspart) was engineered through mutagenesis to create monomeric forms, which are fast acting (Sensh *et al.,* 2010). Conversely, another form of insulin (glargine) was created by mutagenesis to precipitate upon injection and give a sustained release of insulin. Whittingham *et al*., (1997) have reported a crystal structure of prolonged-acting insulin with albumin-binding properties (Sanger and Thompson 1953a,b; Skelton *et al.,* 2001; Vajdos *et al.,* 2001; Boes *et al.,* 2002; Gavira *et al.,* 2002; Teixeira *et al.,* 2002; Weiss *et al.,* 2002; Miyahara *et al.,* 2003; Headey *et al.,* 2004; Hua and Weiss 2004; Fortier *et al.,* 2005; Li *et al.,* 2005; Mark *et al.,* 2005; Sala *et al.,* 2005; Huang *et al.,* 2006; Kuang *et al.,* 2006; Chandrashekaran *et al.,* 2007; Kim *et al.,* 2007; Wan *et al.,* 2008).

Catalytic antibody

Antibodies are proteins that normally bind to a specific molecule but do not alter the bound molecule in any way (Coenraad *et al.,* 2005). A catalytic antibody is a variant of an antibody which has been changed by mutations to have a novel sequence that folds into a structure, resulting into a specific reaction (such as amide bond formation, ester hydrolysis, and decarboxylation). Catalytic antibodies function like enzymes, and are created to catalyze reactions for which there are no naturally occurring enzymes (Paul *et al.,* 1994; Ali *et al.,* 2009). Fifty or more reactions have been made by the action of catalytic antibodies, which were obtained individually by the methods of PE (Branningan and Wilkinson 2002).

Polyketide synthases

Antibiotics such as erythromycin are made by large multidomain proteins called polyketide synthases (Findlow *et al.,* 2003; Chin *et al.,* 2006; Lai *et al.,* 2006; Alekseyev *et al.,* 2007). Site-directed mutagenesis has been used to modify the substrate specificity of the polyketide synthase reaction so that the new product contains a malonate unit, whereas the product of the original enzyme contained a methylmalonate unit (Crawford *et al.,* 2006**)**. In addition to site-directed mutagenesis, the order of the polyketide synthase domains has been shuffled to create proteins that could catalyze the synthesis of new antibiotics (Branningan and Wilkinson 2002).

From basic research to products

It is important to direct basic research to introduce certain products. In fact, the science and market are tightly related to each other by many aspects. Previously a description about industrial (e.g. Protein) and Pharmaceutical approach (eg. Catalytic antibodies) have been presented which have direct application in the Market. However, there are many other examples about basic research, which have been converted to commercial products (Crawford *et al.,* 2006).

From tem-1 *β*-lactamase to GeneEditorTM (Promiga©)

"Apparently, engineering antibiotic resistant gene is not a good idea, however the scientist imagination and innovation enable them to see behind that"

β-lactamase is the resistant factor to the *β*-lactam antibiotics. The plasmid-encoded TEM *β*-lactamase is the most prevalent one in Gram-negative enteric bacteria (Venkatachalam *et al.,* 1994; Matagne 1998).

A discovery of new generation *β*-lactam antibiotics leads to induce new resistant strains that are reported as sensitive. The scientists are typing to understand why microbes have the ability of conversion to a newly resistant form.

Venkatachalam *et al*., (1994) have investigated TEM *β*-lactamase variants with amino acid substitutions in the active-site pocket of the enzyme. The experiments have been identified in natural isolates with increased resistance to extended-spectrum cephalosporins, such as cefotaxime and ceftazidime. Mutants were selected for 100-fold more ceftazidime resistance than wild-type. All mutants had a serine substitution at position 238, a lysine or arginine at position 240, and a small amino acid at position 241. The role of each substitution was investigated by constructing individual G238S, E240K, and R241G mutants as well as the G238, SE240K double mutant. The G238S mutant increases catalytic efficiency for both ceftazidime and cefotaxime. However, to achieve significant increases in catalytic efficiency, both G238S and the E240K mutants are required. The R241G mutant

results in a small increase in catalytic efficiency for only ceftazidime. Using the same strategy the GeneEditor™ *in vitro* Site-Directed Mutagenesis System has been made. This system uses antibiotic selection to obtain high frequency mutants. Selection of oligonucleotides provided the GeneEditor™ System encode mutations that alter the ampicillin resistance gene, creating constructs that confer new additional resistance to the GeneEditor™ antibiotic selection mixture. Mutants generation using this system retain ampicillin resistance and gain resistance to the GeneEditor™ antibiotic selection mixture.

Several research studies have been conducted using GeneEditor™ protocol. Amara and Rhem (2003) have described seven site directed mutations that have been performed in $PhaC_{Pa}$, where 5 conserved residues were replaced by site directed mutagenesis in order to identify the role of these amino acids in catalysis.

CONCLUSION AND FUTURE PROSPECTIVE

PE is a young discipline that has been branched out from the field of genetic engineering. It has been elevated because of the progress in the field of molecular biology. PE is based on the available knowledge about the proteins' structure/function(s), tools/instruments, software, bioinformatics database, available cloned gene, knowledge about available protein, vectors, recombinant strains and other materials that could lead to change in the protein backbone. Protein produced properly from genetic engineering process means a protein that is able to fold correctly and to do particular function(s) efficiently even after being subjected to engineering practices. Protein is modified through its gene or chemically. However, modification of protein through gene is easier. There is no specific limitation of PE tools; any technique that can lead to change the protein constituent of amino acid and result in the modification of protein structure/function(s) is in the frame of PE. Meanwhile, there are some common tools used to reach a specific target. Site directed mutagenesis, which is the first tool that has been used in protein engineering, is still the most favourite one nowadays. More active industrial and pharmaceutical based proteins have been invented by the filed of PE to introduce new function as well as to change its interaction with surrounding environment. His_{tag} proteins are an example (Hoffmann *et al*. 2002). In industrial and medicinal applications, PE has been used to improve the products quality. This is lead to increase the profit of different industrial sectors by yielding safe, efficient, and comfortable products.

PE has a bright future. As applied science, it has been able to solve many complicated scientific points. Improvement in protein applications is the most promising part of PE. As described in this review, the research being done on this filed is significantly important, which can enhance its scientific and technical applications. Scientists working on PE should be scaled, knowledgeable and patient. As an alternative, a scientific group having scientists with different background can lead to successful research findings. PE put great opportunities in the hands of scientists to improve the protein based industry within the frame of technical and pharmaceutical applications. The future brings an increasing interest in using PE and its applications to industrial productions.

ACKNOWLEDGMENT

This work was supported by Deanship of Scientific Research and Research Centre, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. I acknowledge Assistant Prof. Dr. Haider Zaman from Geophysics Department Faculty of Science, King Saud University for some technical advices and revision of this review.

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