Protein Engineering

Many early biotechnology-produced protein drug candidates failed in clinical trials due to their short biological half-life, low affinity for their receptor, or immunogenicity. Recombinant DNA technology has made it possible to engineer specifically altered or novel protein molecules possessing tailored chemical and biological characteristics.

Termed protein engineering, the deliberate design and construction of unique proteins with enhanced or novel molecular properties is a result of specifying the exact amino acid sequence (protein primary structure) of that protein. When applied to enzymes, the process is often called enzyme engineering.

The primary structure affects the protein's conformation. The conformation of each and every amino acid component present in the protein influences the protein's complex 3-D structure. The conformational preference of the protein chain residues determines the protein's secondary structure including a-helices and b-sheets or reverse turns. The local secondary structures are folded into 3-D tertiary structures made up of domains. The domains are not only structural units, but are also functional units often containing intact ligand binding (in a receptor) or enzyme catalytic sites. Thus, protein engineering provides an approach to modify a native protein's structure specifically or to create a unique, new protein with a particular structure.

Protein engineering has numerous powerful theoretical and practical implications for examining and modifying protein structure and function, probing enzyme mechanisms, investigating protein folding and conformation, enhancing protein stability, introducing detectable groups into proteins as an analytical tool, producing improved second generation tailored biopharmaceuticals, and in the case of enzymes, improving catalytic function.

Engineered proteins have been prepared by many different approaches. Direct chemical synthetic routes for small proteins with modified amino acid sequences have been devised using either solution chemistry or solid supports (chemistry occurring while reactants are attached to resin beads) techniques.

Site-Directed Mutagenesis

Site-directed mutagenesis (also called site-specific mutagenesis) is a protein engineering technique allowing specific amino acid residue (site-directed) alteration (mutation) to create new protein entities. Mutagenesis at a single amino acid position in an engineered protein is called a point mutation. Therefore, site-directed mutagenesis techniques can aid in the examination at the molecular level of the relationship between 3-D structure and function of interesting proteins.

Directed Evolution

Today, many of the techniques to engineer proteins with improved properties such as enhanced biological activity, improved catalytic specificity, metabolic stability, etc. are often referred to as "directed evolution". Studying the relationship between a protein's sequence and the resulting protein's property allows for a prediction of the optimal structure-property relationship and thus the "evolved" protein to be synthesized by standard techniques of biotechnology.

Enzyme Engineering

Enzyme engineering is the application of protein engineering techniques to enzymatic molecules. Enzyme engineering can optimize catalytic reactions, improve an enzyme's function under abnormal conditions, and enhance or change the catalytic reaction of unnatural substrates.

An exciting application of protein engineering is the preparation of enzymes that have improved catalytic activity and stability in organic solvents, rather than requiring an aqueous environment. In that case, site directed mutagenesis replaces hydrophilic, charged amino acids and hydrogen bonding residues at the surface of the enzyme with amino acids that stabilize the conformational stability of the protein at the organic solvent–protein surface interface.

Fusion Proteins

Using ligation chemistry to fuse the gene-coding region for one protein with that of another protein, researchers have created chimeric proteins that combine the properties and activities of the two individual parents. The molecule created is called a fusion protein. Fusion proteins contain portions, or the entire amino acid sequences, of both parent proteins. Fusion proteins

have found use in improving the gene expression of a target protein, creating molecules with additive biological activities, and assessing the structure-activity relationships of regions in a protein important to its function.

Antibody Engineering

A pharmaceutically important application of protein engineering is the production of chimeras to examine the structure-activity relationships of a protein. An example is the engineering of humanized or fully human MAbs. These altered MAbs are prepared by expressing a chimeric antibody gene containing the code for both human and murine portions of the resulting antibody protein or the antibody gene for the fully human protein, respectively. The differences between species in the structure-activity relationships and the structure-function relationships of these chimeric or human antibodies can be examined by studying properties such as antigen specificity, affinity, and avidity.

Conclusion

Tremendous advances have occurred in biotechnology since Watson and Crick determined the structure of DNA. Improved pharmaceuticals, novel therapeutic agents, unique diagnostic products, and new drug design tools have resulted from the escalating achievements of pharmaceutical biotechnology. While recombinant DNA technology and hybridoma techniques received most of the press in the late 1980s and early 1990s, a wealth of additional and innovative biotechnologies and approaches has been, and will continue to be, developed in order to enhance pharmaceutical research. Genomics, proteomics, transcriptomics, microarrays, pharmacogenomics/genetics, personalized medicine, metabonomics/ metabolomics, toxicogenomics, glycomics, systems biology, chemical biology, genetically engineered animals, protein engineering, peptide chemistry and peptidomimetics, cell therapy, regenerative medicine, high throughput screening and high-speed combinatorial synthesis are directly influencing the pharmaceutical sciences and are well positioned to significantly impact modern pharmaceutical care. Application of these and yet to be discovered biotechnologies will continue to reshape effective drug therapy as well as improve the competitive, challenging process of drug discovery and development of new medicinal agents and diagnostics. Pharmacists, pharmaceutical scientists and pharmacy students should be poised to take advantage of the products and techniques made available by the unprecedented scope and pace of discovery in biotechnology in the 21st century.

BASIC PRINCIPLES OF GENETIC ENGINEERING

Genetic engineering is the alteration of an organism's genotype using recombinant DNA technology to modify an organism's DNA to achieve desirable traits. The addition of foreign DNA in the form of recombinant DNA vectors generated by molecular cloning is the most common method of genetic engineering. The organism that receives the recombinant DNA is called a genetically modified organism (GMO). If the foreign DNA that is introduced comes from a different species, the host organism is called transgenic. Bacteria, plants, and animals have been genetically modified since the early 1970s for academic, medical, agricultural, and industrial purposes. In the US, GMOs such as Roundup-ready soybeans and borer-resistant corn are part of many common processed foods.

Gene Targeting

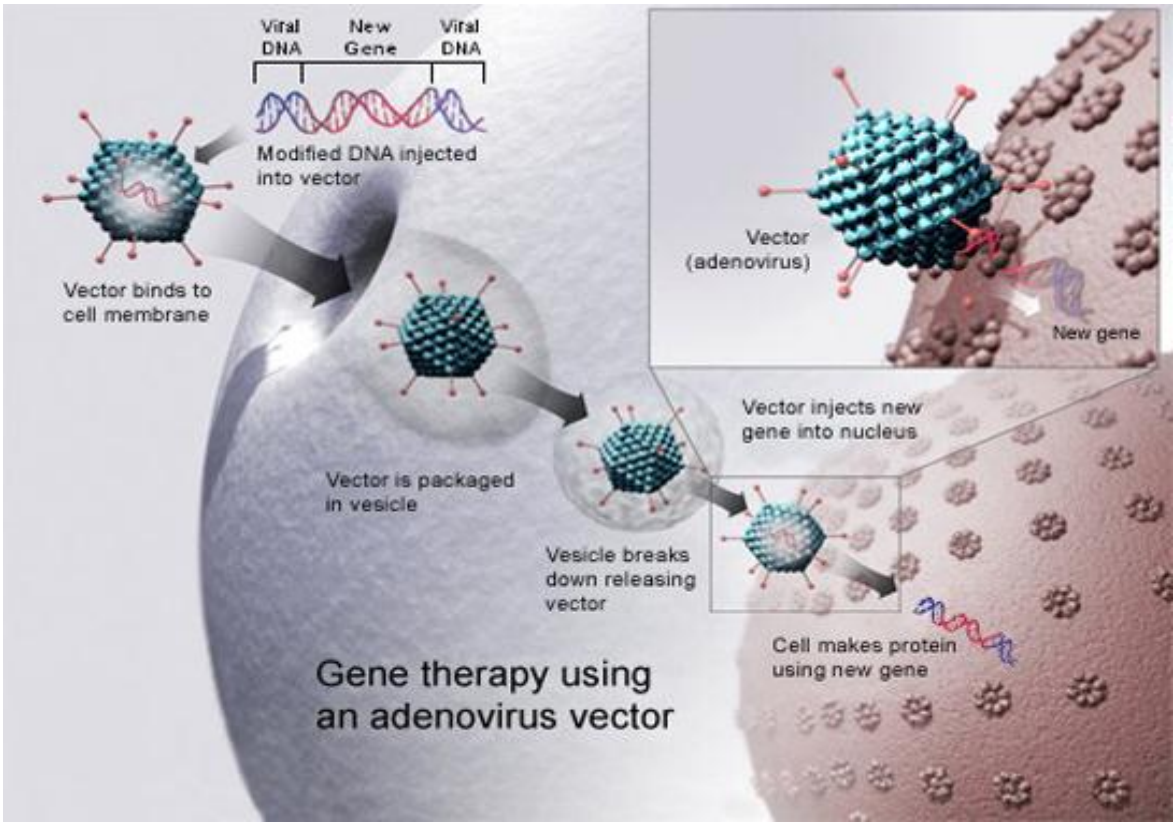
Although classical methods of studying the function of genes began with a given phenotype and determined the genetic basis of that phenotype, modern techniques allow researchers to start at the DNA sequence level and ask: "What does this gene or DNA element do?" This technique, called reverse genetics, has resulted in reversing the classic genetic methodology. This method would be similar to damaging a body part to determine its function. An insect that loses a wing cannot fly, which means that the function of the wing is flight. The classical genetic method would compare insects that cannot fly with insects that can fly, and observe that the non-flying insects have lost wings. Similarly, mutating or deleting genes provides researchers with clues about gene function. The methods used to disable gene function are collectively called gene targeting. Gene targeting is the use of recombinant DNA vectors to alter the expression of a particular gene, either by introducing mutations in a gene, or by eliminating the expression of a certain gene by deleting a part or all of the gene sequence from the genome of an organism.

Genetic Diagnosis and Gene Therapy

The process of testing for suspected genetic defects before administering treatment is called genetic diagnosis by genetic testing. Depending on the inheritance patterns of a disease-causing gene, family members are advised to undergo genetic testing. For example, women diagnosed with breast cancer are usually advised to have a biopsy so that the medical team can determine the genetic basis of cancer development. Treatment plans are based on the

findings of genetic tests that determine the type of cancer. If the cancer is caused by inherited gene mutations, other female relatives are also advised to undergo genetic testing and periodic screening for breast cancer. Genetic testing is also offered for fetuses (or embryos with in vitro fertilization) to determine the presence or absence of disease-causing genes in families with specific debilitating diseases.

Gene therapy is a genetic engineering technique used to cure disease. In its simplest form, it involves the introduction of a good gene at a random location in the genome to aid the cure of a disease that is caused by a mutated gene. The good gene is usually introduced into diseased cells as part of a vector transmitted by a virus that can infect the host cell and deliver the foreign DNA (Figure 1). More advanced forms of gene therapy try to correct the mutation at the original site in the genome, such as is the case with treatment of severe combined immunodeficiency (SCID).



Production of Vaccines, Antibiotics, and Hormones

Traditional vaccination strategies use weakened or inactive forms of microorganisms to mount the initial immune response. Modern techniques use the genes of microorganisms cloned into vectors to mass produce the desired antigen. The antigen is then introduced into

the body to stimulate the primary immune response and trigger immune memory. Genes cloned from the influenza virus have been used to combat the constantly changing strains of this virus.

Antibiotics are a biotechnological product. They are naturally produced by microorganisms, such as fungi, to attain an advantage over bacterial populations. Antibiotics are produced on a large scale by cultivating and manipulating fungal cells.

Recombinant DNA technology was used to produce large-scale quantities of human insulin in *E. coli* as early as 1978. Previously, it was only possible to treat diabetes with pig insulin, which caused allergic reactions in humans because of differences in the gene product. Currently, the vast majority of diabetes suffers who inject insulin do so with insulin produced by bacteria.

Human growth hormone (HGH) is used to treat growth disorders in children. The HGH gene was cloned from a cDNA library and inserted into *E. coli* cells by cloning it into a bacterial vector. Bacterial HGH can be used in humans to reduce symptoms of various growth disorders.