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Medicinal Importance of Plant Proteins

Ankita Sehrawat

Daulat Ram College, University of Delhi, Delhi-110007
E-mail: ankitasehrawat1@gmail.com

Abstract—*Proteins are essential biomolecules required for the normal function, structural maintenance and regulation of the body of all living organisms. Animal proteins and synthetic polymers are being exploited in the medicinal and pharmaceutical industry. Plant proteins are preferred over animal proteins for drug delivery as they have low immunogenicity and can be easily made into fibers, hydrogels and micro- and nano-particles. Zein, wheat gluten and soyproteins are most used plant proteins for bioengineering. Plant proteins also show antifungal activity and prevent the growth of harmful fungi like Aspergillus flavus, Trichoderma viride and Fusarium oxysporum. An anti-fungal protein, chitinase-like protein also shows HIV-1 reverse transcriptase inhibitory activity, indicating to be a potential cure for HIV. Recently, green microalgae, Chlamydomonas reinhardtii has attracted researchers attention to be used as a biomanufacturing host for the production of recombinant proteins, as these microorganisms are safe, easy to transform and inexpensive to grow. Antibodies against herpes simplex virus (HSV) glycoprotein D and antigen 83 (PA83) of anthrax, are produced in C. reinhardtii. Moreover, vaccines against foot-and-mouth disease and classical swine fever virus are also produced in this transgenic alga. Using recombinant DNA technology, cytochrome P450 (a monooxygenase) has been genetically manipulated for the biosynthesis of anti- cancerous (Taxol) and anti-malarial (artemisinin) drugs. In the present review a comprehensive analysis of the medicinal role of plant proteins is highlighted.*

1. INTRODUCTION

Proteins (hormones, enzymes, signaling and antibodies) are essential biomolecules involved in structural maintenance and regulation of the body of all living organisms. DNA transcribes into RNA, and RNA translates into proteins. Inside cell, proteins are produced in the cytosol. Proteins are made of amino acids chains and changes in the arrangement and length of the amino acids determine the function of the protein. Proteins as hormones bind to the receptors on the cell membrane and regulate cell signaling, while as enzymes it catalyzes the metabolic and regulatory reactions and as antibodies it strengthens the immune system.

From ancient times, proteins are being exploited for their medicinal value. For e.g. proteolytic enzymes (papain and chymopapain) from *Carica papaya* fruits are used as digestants, while bromelains from *Ananas comosus* fruit is used as anti-inflammatory agents. With the development of science and technology, the usage of proteins as home remedy in diseases has expanded to large-scale production in the pharmaceutical industry. Earlier animal proteins like collagen and silk were most widely used for medical applications. However, due to the risk of transmission of diseases *via* animals (for e.g. 'mad cow disease' by bovine collagen) researchers suggested plant proteins as an alternative biomaterial in bioengineering. Plants proteins are used for the drug delivery and tissue engineering due to their low immunogenicity and their potential to be made into fibers, nanoparticles and hydrogels.

Plants have antifungal proteins, which not only inhibit the growth of fungi but are also explored for the treatment of human diseases. However, as the concentration of these disease curing bioactive compounds is very low, recombinant DNA technology is used for their bulk production. Traditionally bacterial, fungal and mammalian cell cultures were used for the proteins expression. In the past years, a lot of research has initiated on using plants as a "factory" for protein production. Recently, *Chlamydomonas reinhardtii* was used for the bulk production of antibodies, while CYP450 has been genetically modified for the production of anti-cancerous and malarial drugs. In this review, the importance of plant proteins in medical and pharmaceutical industry will be described with more focus on the use of the plant proteins in bioengineering and recombinant DNA technology.

2. MEDICINAL IMPORTANCE OF PLANT BASED PROTEINS

As biomaterial for tissue engineering and drug delivery

As proteins are the major constituents of the human body, they are preferred over other biomolecules as biomaterial for tissue engineering and drug delivery. Moreover, proteins are more useful in comparison with the synthetic polymers as the later lacks functional groups. Additionally, the presence of amino and carboxyl groups enable proteins to carry different charges depending on the pH. Till now, collagen and silk protein (animal proteins) are most widely used, however these two proteins have several disadvantages like poor degradation rate and high immunogenicity, which demands for a better biomaterial for medical practices. Zein (*Zea mays*), soyproteins (Sorghum) and wheat proteins, gluten, gliadin and glutenin (*Triticum aestivum*) are the major plant proteins, which could be used as a biomaterial [1]. As these proteins have higher net negative charge, therefore, are more suitable for delivering positively charged drugs. Additionally, plant proteins are rich in polar amino acids therefore, attract cells easily. Hydrogels (three-dimensional, cross-linked networks of water-soluble polymers) prepared using soyproteins and PEG is used for moist wound dressing applications. Zein films are used to culture human liver cells (HL-7702) and mice fibroblasts (NIH3T3). Zein microspheres are used to deliver drug ivermectin to cure demodicidosis (a parasitic disease) in dogs.

These studies showed the advantages of plant proteins over animal proteins and synthetic polymer for drug delivery. However, the biocompatibility of plant proteins *in vivo* still needs to analyzed in details.

2.1. Anti-fungal proteins with novel functions

Pathogenic fungi decrease the growth potential of humans, animals and crops and causes enormous economic losses. All living organisms have innate immune/defense response system for inhibiting fungal invasions by producing antifungal proteins. Plant antifungal proteins includes chitinases and chitinase-like proteins, chitin-binding proteins, lectins, defensins and defensin-like proteins, glucanases, lipid transfer proteins, peroxidases, protease inhibitors, ribosome-inactivating proteins, storage 2S albumins and thaumatin-like proteins [2]. In addition to antifungal activity, these proteins also have medicinal and pharmaceutical value. For e.g. chitinases and chitinase-like proteins have stimulatory effects on macrophages and splenocytes, therefore has a role in increasing immunity. Similarly, chitinase-like protein from *Panax notoginseng* (a medicinal plant) shows HIV-1 reverse transcriptase inhibitory activity, indicating a potential cure for HIV.

Sesquin, a defensin-like peptide of *Vigna sesquipe* inhibits the growth of leukemia M1 and breast cancer MCF-7 cells and reduces the activity of HIV-1 reverse transcriptase [3]. It also shows antifungal activity towards *Botrytis cinerea*, *Fusarium oxysporum* and antibacterial activity towards *Escherichia coli* and *Bacillus megaterium*.

Lectins being carbohydrate-binding protein inhibits fungal growth by interacting with fungal cell wall (composed of glucans, chitin and other sugar polymer). Jacalin-related lectins (JRL) are galactose- and mannose-specific members of lectin family. Helja (a JRL from *Helianthus annuus* seedlings) shows inhibition against human pathogenic yeast like *Candida tropicalis, C. parapsilosis, C. albicans and Pichia membranifaciens* [4]. It is proposed that JRL induces reactive oxygen species (ROS, superoxide radicals, hydrogen peroxide, single oxygen and peroxynitrite) production in the harmful yeast cells, and inhibits their growth. Similarly, lectin from *Pisum sativum* seeds inhibited the growth of *Aspergillus flavus, Trichoderma viride and Fusarium oxysporum* [5]. Chitin-binding lectin from *Schinus terebinthifolius* leaves inhibits *Escherichia coli, Klebsiella pneumoniae and Salmonella enteritidis* growth. Lectin from *Phaseolus vulgaris* shows anti-proliferative activity against leukemia cells. However, it has no inhibitory activity against HIV-1 reverse transcriptase or mitogenic activity toward mouse splenocytes [6], indicating differential roles of antifungal proteins and their diverse application in the pharmaceutical industry.

These studies showed the role of plant proteins not only in inhibiting fungal growth but also suggested their practical applications in controlling diseases.

2.2. Recombinant proteins with plant based origin

Recombinant proteins are most commonly used for industrial and medical applications. Most commonly used host for the recombinant protein expression are bacteria, yeast, plants, mammalian cell lines and transgenic animals. Recently, green microalgae has attracted attention of researchers to be used as a biomanufacturing host for the production of recombinant proteins. Algae are safe, easy to transform and inexpensive to grow [7]. *Chlamydomonas reinhardtii* is the most widely used host for biomanufacturing of the recombinant proteins and small molecules for industries such as bioenergy, biopharmaceuticals, biomaterials, agriculture and cosmetics. *Chlorella*, *Scenedesmus* and *Dunaliella* are also shown to have great potential for the large-scale production of biomolecules. The first mammalian protein production in algae was human monoclonal antibody against herpes simplex virus (HSV) glycoprotein D [8]. Antibodies against antigen 83 (PA83) of anthrax was produced in *C. reinhardtii* [9]. Recently, *C. reinhardtii* chloroplast were used to produce immunotoxin, anti-CD22-exotoxin A, which specifically kill CD22 positive Burkitt's lymphoma cell line and improved mouse survival [10]. These examples showed a new way of producing fusion proteins of medicinal value. Human metallothionein-2 gene product, expressed in the chloroplast of *C. reinhardtii* was shown to provide resistance to UV-B exposed cells [11]. Allophycocyanin (APC), a photosynthetic antenna protein in cyanobacteria and red algae, inhibited S-180 carcinoma in mice. Transgenic algae have also been exploited for vaccines production for foot-and-mouth disease virus and classical swine fever virus [12].

Cytochrome P450s are monooxygenases and involved in the biosynthesis of hormones, defensive secondary metabolites (phytoalexins), phenylpropanoids, terpenoids, fatty acids and xenobiotics [13]. These are the prime targets in the metabolic engineering approaches and secondary metabolites production of medicinal drugs like taxol or indole alkaloids. Plants contain many useful chemotherapeutic agents, however their amount is very less. Therefore, to increase their production a comprehensive analysis of the biosynthetic pathway, the enzymes involved in it and the genes encoding these proteins is required. Cytochrome P450 has been manipulated for drug biosynthesis. For e.g. taxol (treat carcinomas, melanomas and sarcomas) is derived from the bark of the Pacific yew (*Taxus brevifolia*) in very low amounts. Using genetic engineering, production of taxol could be increased by genetic manipulation of CYP450 genes (a key enzyme in the biosynthetic pathway of taxol). Similarly, artemisinin (an anti-malarial drug) is produced by introducing specific genes encoding for amorphadiene synthase and CYP71AV1 from *Artemisia annua*. Such approach has showed a new method for the bulk synthesis of drugs. These results showed plant CYP450 as the refined "chemical factory" for the synthesis of drugs with very low concentrations in plant.

2.3. Commercially valuable plant proteins

In addition to importance of proteins in structural maintenance of the human body, few plant proteins are used commercially as bioactive compound. For e.g. papain and chymopapain, enzymes extracted from *Carica papaya* fruit are used as digestants [14]. Bromelains (milk-clotting enzymes) extracted from *Ananas comosus* fruit juice are used as anti-inflammatory agents. Ficin is a proteolytic enzyme from Ficus latex. Miraculin [*Synsepalum dulcificum* (Miracle fruit)], monellin and thaumatins are intensely sweet proteins used commercially as sugar replacements. *Curcuma longa* (turmeric) rhizome contains TAP (turmeric antioxidant protein). TAP prevents thiol (SH) depletion and inhibits Ca-ATPase from inactivation. TAP maintains antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) levels under stress conditions, therefore helps in ROS detoxification and prevents oxidative damage in the cell [15].

3. CONCLUDING REMARKS

From ancient times, plant proteins have been used for the treatment of human diseases. With recent advancement in science, plants are now exploited not only for the production of bioactive compounds but also for the production of antibiotics, vaccines and antibodies. Bulk production of anti-cancerous and anti-malarial drugs could be performed in the plants by modulating key enzyme of the biosynthetic pathway of these drugs. In comparison with animals, plants are relatively safe for the drug delivery and hydrogel preparations as they are cytocompatible, less immunogenic and biodegradable.

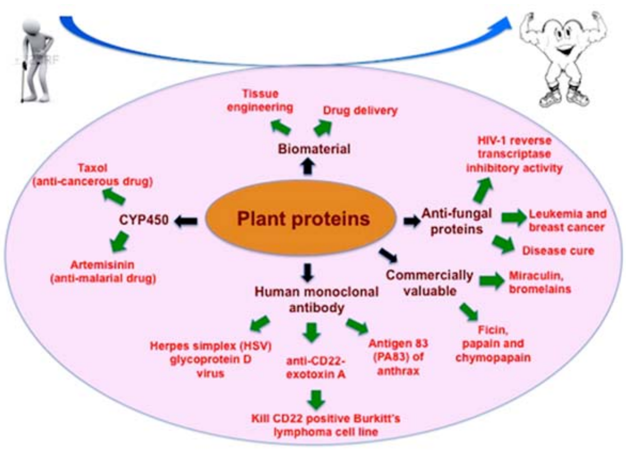

Fig. 1: Application of plant proteins in medicine.

Described examples in the present review clearly showed that plant proteins have a great potential in biomedical and pharmaceutical industry (Fig. 1) However, only few plant proteins including zein and soyproteins have been used for *in vivo* studies, therefore future research should be done on other proteins before replacing them with currently available animal and synthetic material for bioengineering.

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