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Zein-the biopolymer, properties, preparation and applications

V Gomathi[1], Reshmi Krishna A[2*]

[1] Professor, Department of Pharmacology, Vinayaka Mission's College of Pharmacy, Vinayaka Mission's Research foundation (Deemed to be University), Ariyannur, Salem, Tamil Nadu, India

[2] Research Scholar, Department of Pharmacology, Vinayaka Mission's College of Pharmacy, Vinayaka Mission's Research foundation (Deemed to be University), Ariyannur, Salem, Tamil Nadu, India

Abstract
Biopolymers are natural polymers produced by living organisms and microbes. They are produced from natural and renewable resources and usually falls under three categories - polynucleotides, polypeptides and polysaccharides. Biopolymers have great applications in medical, consumer and food industries. Polysaccharides and their synthetic and semisynthetic derivatives are the most widely used biopolymers. But nowadays, polynucleotides and polypeptides are also widely explored due to advancements in tissue engineering and macromolecular self-assembly techniques. The advantages of being biodegradable, biocompatible with lower immunogenicity and greater potential for biomimicry, makes biopolymers the most suitable candidate for drug delivery applications. In this review, the biopolymer polypeptide from maize- zein is studied for its excellent properties as drug carrier. Zein, the prolamine protein found in the endosperm of corn is amphiphilic in nature and finds several applications in forming microcapsules, nanostructures, films, discs, spheres due to its mesophase behaviour in ethanol-water mixture. Zein is a GRAS compound possessing hydrophobic behaviour making it suitable for preparing nanoparticles encapsulating bioactive compounds. The self-assembling behaviour of zein when subjected to change in polarity, helps to readily encapsulate necessary compounds quite easily.

Keywords: zein, biopolymer, biocompatibility, biodegradability, nanoparticles

Introduction

Zein is a normally occurring plant protein with an isoelectric pH of 6.2. It comes under the group of prolamins and is made out of high amounts of hydrophobic amino acids. The molecular weight of zein is around 25-40 kDa. It is classified into four types based on molecular weight α-zein (19 kDa & 22 kDa), β-zein (14 kDa), γ-zein (16kDa and 27 kDa), and **δ**-zein (10 kDa) Zein protein is amphiphilic in nature but because of higher hydrophobic amino acid content, it is insoluble in water but dissolves in ethanol, acetone or acetylacetone, and solutions with pH ≥ 11. Zein is rich in glutamic acid, leucine, proline and alanine. Its hydrophobic nature and poor solubility in water are mainly due to the high proportion of non-polar amino acids (leucine, proline and alanine). They are deficient in basic and acid amino acids [1]. There are two types of zein available: yellow zein and white zein. The yellow zein consists of high concentrations of xanthophyll pigments and white zein is obtained by the decolourisation of yellow zein and has very less xanthophyll content [2]. Zein has antibacterial properties and can give a very tough coating on substances and for this reason, it is widely used in food and pharmaceutical industries. Zein protein is resistant to the gastric environment and also exhibit mucoadhesive properties, so a suitable candidate for gastroretentive drug delivery system. It is suitable for mucosal delivery of drugs and vaccines and also can be combined with other potential polymers to form gel type dosage forms for the delivery of biologically active compounds. When formulated as nanoparticles, the specific structure of zein protein and its hydrophobicity contributes to the utilization of zein in the body by phagocytosis and hence can influence the immune system too [3].

Zein in nanotechnology

The size range of nanomaterials (1 to 100 nm) offers larger surface area per volume. A nanocomposite can be smaller than a bacterial cell, close to the size of proteins and even to the size of a DNA strand which can be 2 to 3 nm, Zein has great application in nano drug delivery and can also be used as composite in preparing drug packing, food, nutrients, pesticides and even in preparing bandages and for wound treatment [4]. Zein is highly heat, moisture, abrasion resistant, which makes it most suitable as a coating material and provides longer shelf life for the product formulated. Zein based films, fibers and gels, controlled drug delivery systems as tablets or microparticles or nano particles are finding vast applications in various fields.

Zein based controlled drug delivery systems

Controlled drug delivery offers greater therapeutic effectiveness from the loaded carriers. Many anti- tumour drugs have been incorporated in zein for controlled drug delivery [5, 6]. Several conjugated zein/polysaccharide preparations have been developed for many antitumour drugs. Zein microspheres loaded with anti-parasitic drug

Ivermectin has been developed for the subcutaneous and oral use for dogs [6]. Zein microspheres has great applications in scaffolds preparation and tissue engineering. Zein based microspheres have been successfully loaded with cardiotonic glycosides [7]. If the particle size is appropriate, site targeting can also be considered. Zein can also be used as a matrix in monolithic controlled release tablets [8].

Zein as coating material

Zein provides fast and easy coating which is resistant to abrasion, moisture and heat. Before the coating was used to mask the unpleasant taste of the drug or to improve the overall appearance [9]. Zein coated tablets showed improved hardness when compared to CAP or HPMC. Usually, propylene glycol is used as a plasticizer and parabens as preservatives [10].

Zein based films

Oleic acid based zein film found to be insoluble in gastric fluids while readily soluble in intestinal fluids. Many studies also showed the biocompatibility of zein films with endothelial cells of umbilical vein and platelets. Zein film and heparin loaded zein film suppressed platelet adhesion and has better anticoagulation effect. Both phase separation and solvent evaporation techniques can be fused for preparing films. Glycerol when added as a plasticizer, decreased the tensile strength and stiffness of the film. The dissolution profile also showed differences with the quantity of glycerol in the films [11].

Zein- gel based systems

Zein can be used as a polymer for forming gel-based systems which function in situ, thereby extending the local releasee of the drug. The in- situ gel formation also improved the anticancer activity of many drugs [12].

Zein in vaccine delivery

Zein can be used in vaccine delivery because of the strong mucoadhesive property and its protection for antigens from the drastic oral environmental conditions [13]. This provides a new route for non-invasive vaccine delivery using zein as a carrier. One of the studies revealed that the release of antigen occurred for more than 7 days. More studies are needed in this regard including the non-invasive nasal route [14].

Zein in scaffolds and fibers in tissue engineering

Tissue engineering is widely used in regenerative medicine nowadays. In this we prepare scaffolds in which the tissues are incorporated.

Zein has proved its use preparing such scaffolds and films with particle of diameters ranging from 100 to 2500nm for culturing human liver cells. Nanofibers prepared by incorporation of tannin in zein were prepared by electrospinning. Scientists also prepared mucoadhesive ultrafine composite fibers by electrospinning [15].

Methods of preparing protein nanoparticles

The preparation of protein nanoparticles depends on the attractive and repulsive forces and the resultant conformational changes depending on the concentration, composition, and the conditions used like ionic strength, pH and type of solvent used [16].

Coacervation/Desolvation process

Coacervation process is based on the difference in solubility of proteins in solvents in relation to its ionic strength, solvent polarity, pH and presence of electrolytes. In this method, the protein is made into a colloidal form by extracting into an antisolvent from a homogenous solution form being present in the solvent. The presence of antisolvent leads to confirmational changes in the protein structure and desolvation occurs. After a certain level of desolvation, precipitation occurs. The formed nanoparticles are then crosslinked with glutaraldehyde. The amount of the antisolvent used and pH of the solution greatly affects the size of the nanoparticles formed. Higher pH and higher salt concentration always favour formation of faster and smaller nanoparticles [17]. After desolvation, to prepare a nanosuspension, a resolvating agent can be used.

Emulsion /solvent extraction

The protein nanoparticles were prepared by preparing an emulsion using a homogenizer. The nanoparticles are formed at the water oil interface. Various surfactants can be used for producing nanoparticles eg: phosphatidylcholine, span 80 etc. After formation of nanoparticles, crosslinking can be done using glutaraldehyde or heating at 60 ^{o}C for 20 mts. The particles are then centrifuged and washed with organic solvents. We get larger particles by this method when compared to particles obtained by coacervation method. Emulsion/solvent extraction method involves two steps-Emulsion diffusion and emulsion solvent evaporation. In the first step, the protein is dissolved in the oil phase at high stirring stress and drug is added. The solution is stabilized by adding stabilizing agent and the drug entrapped particles are separated by precipitation. In the subsequent step, the protein is dissolved in solvent and the solution is emulsified with drug under high shear in the presence of a stabilizer. The solvent is then removed by vacuum. The protein nanocarriers are thus prepare by precipitation [18].

Complex coacervation

This is a method ideally suited for entrapment of DNA in gene. The amphoteric nature of protein facilitates formation of charged particles by adjusting the pH of the solution. The charged particles bins with the polyelectrolytes through electrostatic attractions, which helps to deliver the DNA/oligonucleotide entrapment into nanoparticles [1].

Electrospray method

This is a new method for preparation of protein nanoparticles. The process consists of taking protein solution in a syringe connected to an electrode connected to a power source. An oppositely charged metal foil electrode is placed on the opposite side and termed as collector. When the solution enters the electric field from the tip of the syringe, it forms a cone because of surface tension and finally breaks into droplets in the high electric field to even nano level [19]. Drugs and nucleic acids could be easily incorporated into the prepared protein NPs using this technique.

Nanoprecipitation

This method is based on solvent displacement through two steps. First is the electrospray technique of polymer solution to form particles, followed by nanoprecipitation. The NPs

were synthesized by a rapid diffusion of the protein solution into the solvent, which increases the surface tension at the interface between the two liquids, causing an increase in surface area and leading to precipitation of the protein nanocarriers [4]. This process can be with or without surfactants. It's a simple, rapid, reproducible process.

Dialysis

This is a method very similar to the nanoprecipitation. Here a dialysis membrane bag is used to carry the protein solution and in that there is gradual loss of solubility of the proteins leading to the formation of nanoparticles. A non-solvent like water is used as outer phase against the dialysis membrane. The organic solvent is slowly replaced by water, leading to the formation of nanoparticles as suspension. The size and structure of the nanoparticles are affected by the type of solvents used. Drugs are encapsulated within the protein nanoparticles [19].

Concluding remarks

Zein is a versatile biopolymer which is yet to be explored to its maximum potential. The amphiphilic and hydrophobic characteristics of zein makes it a suitable candidate for drug delivery.

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