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Pichia Pastoris Manufactures Polymers that are Composed of Proteins

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Abstract

Applications in biomedicine, pharmaceutics, and diagnostics are driving interest in the specific coupling of de novo created recombinant protein polymers for the creation of precisely shaped nanomaterials. The genetic design of the protein polymers can be improved by using specially interacting peptides. The binding of specific proline-rich ligands by so-called WW-domains is an illustration of such an interaction. In this work, we looked at the production of these domains in the yeast Pichia pastoris as components of otherwise non-interacting protein polymers, as well as whether combining them would result in polymer coupling.

Keywords: Block copolymers; Collagen; Elastin; Gelatin; Pichia pastoris; Protein expression; Protein-based polymers; Proteolysis; Self-assembly; Silk

Introduction

Over the past three decades, the creation of so-called protein-based polymers has brought together the fields of materials science and genetic engineering. These proteins, which frequently have repeating amino acid sequences, possess the physical qualities necessary for their usage as functional materials. Collagen, silk, and elastin are well-known examples found in nature, but man-made sequences have also been developed. Recombinant DNA technology can be used to manufacture these proteins in an appropriate host. This class of polymers is particularly interesting to the disciplines of nanomaterials and biological research due to the inherent control over monomer sequence and molecular size [1,2]. Due to its frequently high yields and possible advantages for bioprocessing, the methylotrophic yeast Pichia pastoris is replacing Escherichia coli as the primary workhorse for the manufacture of these polymers. Here, we give a summary of the protein-based polymers made by P. pastoris. We list their physicochemical characteristics, briefly touch on potential uses, and then go into detail on their biosynthesis. The following are some difficulties that could arise while employing P. pastoris to make polymers: Proteolytic degradation, in vivo self-assembly, and (i) low yields and poor process control in shaking flask cultures, i.e., the requirement for bioreactors. We go over solutions to these problems in the hopes that they would be useful to readers who are generally interested in protein expression in P. pastoris [1,3].

A form of nanostructure known as polymer-protein hybrids is made up of protein-polymer conjugates, or complexes with one protein joined to one or more polymer chains. Since many proteins are created by the body naturally and are thus well tolerated and metabolised, the protein component typically offers the benefits of biocompatibility and biodegradability. Despite being employed as targeted therapeutic medications, proteins still have two major drawbacks: a lack of stability and insufficient circulation times. To further improve pharmacologic behaviour and stability, protein-polymer conjugates have been studied. Polymer-protein particles having distinctive shapes and activities, such as stimuli responsiveness, enrichment in particular tissue types, and enzyme activity, can be created by modifying the chemical structure of the protein-polymer conjugates. Recent research has concentrated on polymer-protein particles due to their potential use in gene and medication delivery, bioseparations, imaging, and biosensing [4,5].

Making polymers with a regulated monomer sequence has been a long-term goal in materials science. Although synthetic chemistry has advanced, natural sequential polymers like DNA and proteins exhibit an unmatched amount of control. These biological macromolecules have a predetermined molecular size and a predetermined arrangement of the monomers of nucleotides or amino acids. Proteins acquire distinctive features when they fold into a three-dimensional structure according to their main sequence. Nature has produced an astounding variety of proteins from 20 different amino acid monomers, including enzymes, antibodies, peptide hormones, and proteins with a structure-forming, viscoelastic, or colloidal function [6,7].

The skeletons and structures of cells, tissues, and organisms are mostly shaped by structural proteins, such as silk fibroins. When compared to the amino acid sequences of functional proteins like enzymes and antibodies, structural proteins frequently display distinguishing characteristics, such as a repeating tandem motif. In recent years, structural protein-based materials have been investigated and developed as biological, clothing, and structural materials. The definition of structural proteins, techniques for classifying structural proteins as polymeric materials, and prospective applications are all covered in this paper.

The 20 natural amino acids that make up proteins are connected together by amide bonds. In addition to the 20 naturally occurring amino acids, other amino acids, such as L-3,4-dihydroxyphenylalanine (DOPA), hydroxyproline (Hyp), dityrosine, and selenomethionine, are not produced by ribosomes directly. These amino acids are produced through posttranslational modifications. These amino acids and nonribosomal peptides frequently have a significant impact on both the structure and functional proteins. Many times, the amino acid sequence of structural proteins repeats to create a higher-order structure through intermolecular and/or intramolecular hydrogen bonding. One illustration is the disulfide link that can lead to protein dimerization and hierarchical structures when it forms between two cysteine residues. It is challenging to distinguish a structural protein just based on the sequence or structure because, generally speaking, the

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amino acid sequence and the structure that is created rely on the type of structural protein. especially when non-natural sequences produced by chemical synthesis and gene recombination procedures are taken into consideration, Even harder and trickier is defining a structural protein. A structural protein is therefore "a protein that possesses a characteristic amino acid sequence or motif that repeats and forms a skeleton or contributes to the mechanical properties of a living organism, cell, or material" (Fig. 1) in this topic review. Proteins that are structural can be globular or fibrillar in nature. These motifs typically create higher-order structures through intra- or intermolecular interactions, resulting in the manifestation of physical properties. However, the requirement of a hierarchical structure should not be included in this description when taking into account protein-based amorphous materials [8,9].

Discussion

The average human body cell is around 1,000 times larger than a nanoparticle, which is a particle with a diameter of 1 to 1000 nanometres. They are excellent drug delivery systems because of their small size, flexible construction, and high surface-to-volume ratio. Metals, polysaccharides, and proteins are just a few of the components that can be used to create nanoparticles. Biodegradability, bioavailability, and comparatively low cost are advantages of biological protein-based nanoparticles such silk, keratin, collagen, elastin, maize zein, and soy protein-based nanoparticles. Numerous protein nanoparticles are simple to handle and can be altered to meet desired criteria such size, shape, and weight. Many materials that are not biocompatible and have a detrimental effect on the environment are being replaced with protein nanoparticles in a number of contexts. Here, we make an effort to examine the literature on protein-based nanoparticles with a focus on their use in the biomedical and drug delivery domains. Additional information is offered on controlling nanoparticle properties, particular protein nanoparticle applications, and fabrication processes.

The development of genetically produced polymers with excellent control over monomer sequence and polymer length is the result of improvements in recombinant technology. These polymers have potential for use in medication delivery since it is possible to analyse how precise architectures relate to function. Many relevant polymers for drug delivery have been produced to date using chemically derived and developed methods of synthesis, including some that are now being utilised in patients. They do, however, have shortcomings, such as those associated with statistical characterisation of traditional polymer synthesis processes. The precise order and accuracy of amino acid residues can be achieved through genetic coding, and producing such recombinant polymers in living things enables the development of monodisperse polymers with particular functions and physical features. In order to take advantage of the physicochemical features of structural proteins, research into elastin-like, silk-like, and silk-elastinlike protein polymers, for instance, has resulted in the creation of delivery systems based on natural themes of structural proteins. Moreover, protein-based polymers based on additional natural motifs and de novo designs are beginning to yield promising structures for drug and gene delivery applications where precise control over structure promises connection with function and directs the development of new and improved constructions. Recombinant polymers have not yet been utilised in clinical applications for the delivery of bioactive substances. The development of secure and efficient systems for use in the clinic can be guided by the lessons acquired from fundamental research with these polymers. This tutorial overview summarises achievements in the design and application of recombinant polymers for medication and gene delivery, and it examines difficulties and potential future uses of these polymers.

Conclusion

P. pastoris has become a desirable eukaryotic alternative to the bacterium E. coli, which has historically been the major microbial workhorse for the creation of protein polymers. Many of the protein polymers expressed in P. pastoris have been successfully generated as secreted proteins because the repetitive genes generating protein polymers are stably incorporated into the yeast genome without the need for selective pressure. The primary benefit of secretory production is that it offers a first, extremely efficient purifying stage. This is a crucial consideration in the field of protein polymer research because it costs a lot of money to characterise or test the applicability of the resulting supramolecular materials. Large volumes of protein polymers are frequently generated utilising bench-top bioreactors with only minimal purifying work. Furthermore, straightforward downstream processing is crucial for eventual economic viability.

It is obvious that there is no one perfect host for all proteins. However, P. pastoris does appear to be particularly effective for gelatin-like proteins and octapeptide repeats that are inspired by silk, as it consistently yields g/L levels for the majority of these polymers. Spider silks can be manufactured effectively, but only in an intracellular form. Since hydrophobic ELPs seem to self-assemble during secretion, P. pastoris currently looks less appropriate for the creation of these proteins, even though elastin-like proteins with high transition temperatures are produced efficiently. It would be worthwhile to investigate the intracellular expression of hydrophobic ELPs in P. pastoris in this regard. Although the nonsecretion of trimeric collagen has not yet been resolved, it has been shown that adequately modified P. pastoris is capable of precisely hydroxylating human collagenous sequences.

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