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Poly-Glutamic Acid (PGA) — Structure, Synthesis, Genomic Organization and Its Application: A Review

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***Abstract*— Mankind has been exploring many new dimensions and techniques to obtain the most feasible and cost effective biopolymers. Biopolymers are the 21st century polymers and are in great demand as they are very easy to produce through the microbial partners and their conglomeration. Future is all about the emancipation of higher finesse quality products with an iota of eco-friendliness. Biodegradation and low pollutant output has made these polymers from the biological sources, a great eco-friendly substituent for the chemical based polymers. Poly-γ-Glutamic Acid (PGA) is a naturally occurring anionic polymer composed of extensively viscous homo-polyamide of D and L-Glutamic acid units. Its multi-functionality, biodegradability, non-toxicity, compatibility and edibility have made it a promising biopolymer for food, cosmetics and pharmaceutical industries. PGA has several applications in environmental, agricultural and biomedical products as well as it can be used as biodegradable packing material and in other applications including conductive display material, drug delivery, gene vector, dispersant and enzyme-immobilizing material. In this review, we have discussed about the history of PGA, its structure, synthesis, genes and its organization and the applications of PGA.**

***Index Terms*— Poly Glutamic Acid, Hydrogel, Solid State Fermentation, Submerged Fermentation, Biocementation.**

I. INTRODUCTION

Last few decades witnessed the development of several biopolymers which are earth friendly biodegradable polymers for several applications in comparison to those polymers which are derived from petroleum products. Some of these biopolymers belong to Poly-amide family. Poly-amides are enlarged family of amide linked monomeric chemical compounds, having either homogeneous or heterogeneous substituted molecule, constitutes up an intriguing and naturally degradable biopolymer.

The bulk of polyamides are the co-polymeric proteins. A small group of polyamides are referred to as poly amino acids in order to distinguish them from the proteins due to different features of its biosynthesis. Poly-amino acids are composed of monomeric units of amino acids, at least in the backbone as compared to proteins which are composed by hetero-oligomeric units of amino acids. Proteins are biosynthesized from DNA, through transcription and ribosomal mediated translation mechanism whereas the biosynthesis of poly-amino acids are independent of transcription and translation machinery.

Naturally, three different types of poly-amino acids exist, i.e., Poly-γ-Glutamic acid (γ-PGA), Poly-ε-lysine (ε-PL) and Cyanophycin. PGA is a naturally occurring, infrequent anionic polymeric compound composed of extensively viscous homo-polyamide of D and L-Glutamic acid units and it is an extracellular polymer which is completely biodegradable and non-toxic to humans.

II. HISTORY AND STRUCTURE

A. History

PGA was first discovered in 1973 by Ivanovic and his co-workers in the form of capsule in Bacillus anthracis, a sporulative Gram-positive bacterium and the causative agent of Anthrax. PGA has been reported to be the major component in the viscous sticky mucilage of Natto, a health food in Japan, which has been in

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domestic and commercial use for more than a thousand years, produced by steaming small soybeans and fermenting them with starter culture of Bacillus subtilis (natto).

B. Structure

Poly-γ-Glutamic acid (PGA) is a homo-polyamide consisting of D- and L-Glutamic acid monomers, which are interlinked by the amide linkages formed between the α-amino and the γ-carboxyl groups and therefore it is resistant to proteases. PGA is an optically active biopolymer having a chiral centre in every glutamate unit. Three types of PGA are active stereo chemicals: the homopolymer composed of D-Glutamate (D-PGA), the homopolymer of L-Glutamate (L-PGA), and the copolymer composed of D- and L-Glutamate (D-L-PGA).

C. Physical and Chemical Properties

γ-PGA is water-soluble and anionic polymer; however its free acid form (H+) is insoluble in water, whereas the salt forms like K+, Na+, NH4+, Ca2+ and Mg2+ are completely soluble in water. It is completely biodegradable and nontoxic to humans. It acts as a natural moisturizer and its hygroscopic and moisturizing effects were comparable with Hyaluronic acid. Cross-linked γ-PGA is one of the most useful Super Absorbent Polymers (SAP). The molecular weight of PGA ranges from 100 KDa to 2,500 KDa.

III. ORGANISMS PRODUCING PGA AND BIOSYNTHESIS

A. Organisms Producing PGA

Several microorganisms produce PGA which includes many varied species of bacteria, one archaea and one eukaryotic organism. Some of the organisms which produce PGA are B. anthracis, B. subtilis, B. licheniformis, B. megaterium, Bacillus pumilus, Planococcus halophilus, Sporosarcina halophile, Staphylococcus epidermidis, Natrialba aegyptiaca and Hydra. It has been observed that only Gram positive bacteria produce PGA.

B. Biosynthesis of PGA

The synthesis of γ-PGA is a ribosome independent process which is catalysed by enzymes and it occurs in two steps in bacteria: First step is the synthesis of D and L-Glutamic acid and in the second step, D and L-Glutamic acid units join to form PGA. For the production of γ-Poly-Glutamic acid, L-Glutamic acid serves as an inducer and feed stock. Both D- and L-Glutamic acid optical enantiomers exist in the γ-PGA produced by B. subtilis, mostly in the ratio of 50-70% of D-Glutamic acid to 50-30% of L-Glutamic acid.

C. Genes Involved in Biosynthesis

Bacillus group of bacteria (like B. subtilis 168, B. subtilis (natto), B. licheniformis, B. anthracis) and S. epidermidis, possess PGA synthesizing genes. The PGA synthesis genes in B. anthracis is carried out by the plasmid pXO2. Four genes, capB, capC, capA and capE are necessary for the Polyglutamate synthesis. In B. subtilis, three genes (pgsB, pgsC and pgsAA) were initially identified as involved in Polyglutamate synthesis.

IV. PRODUCTION OF Γ-PGA

Production of PGA can be carried out by solid state fermentation (SSF) or by submerged fermentation (SmF). SSF technique provides higher yields and has many numerous advantages. Cereal based substrates (like beans, corns, wheat, soybeans, rice, millets etc.) which were traditionally used are still favoured along with the non-traditional substrates like wastes from agricultural source, forest and food processing units.

Depending on the strains of microorganisms used, the media requirements and culture conditions vary for PGA production. The PGA producing bacteria are classified into two groups as Glutamic acid dependent bacteria and Glutamic acid independent bacteria. In most of the fermentation processes 20-30 g/L of L-Glutamic acid was utilized for the production of PGA.

V. APPLICATIONS OF PGA

A. Biodegradable Substitutes

PGA and its derivatives can be used as thermoplastics, fibres, films and membranes. Although PGA is a novel nylon (polyamide plastic), under ambient humidity it does not act as a thermoplastic. Recently, Chung et al. 2013, designed a new protein based biopolymer responsive system through temperature modification or solvent interaction which changed its structure, geometry and chemistry.

B. Hydrogels

Hydrogel is a semi-rigid jelly-like colloid. Cross-linked γ-PGA is one of the most useful super absorbent polymers (SAP). Microscopic examination of the PGA hydrogel reveals a multi bag like structure that enables it to absorb moisture 5000 times than its own weight. The amount of water contained within the PGA hydrogel is influenced by pH and salt content.

C. Drug Delivery

There are several reports which suggest that PGA can be used as a drug carrier. PGA paclitaxel (PG-TXL), prepared by covalent bonding of native Paclitaxel to a Glutamic acid polymer, is a water soluble form of Bristol-Myers Squibb's well known cancer drug Taxol. Preclinical data suggested that the uptake of PG-TXL by tumour cells was ~5 fold greater than that of Paclitaxel when equivalent doses were used.

D. Applications in Food Industry

In food industry γ-PGA has an inordinate diversity of applications. γ-PGA can be used as a bitterness relieving agent and for prevention of aging and improvement of textures of starch-based bakery products, noodles and stabilizer for ice cream. Low concentrations of γ-PGA were testified to mend the taste and drinkability of juices and other drinks.

E. PGA in Agriculture

Wang et al. 2008, proved that lipopeptides and PGA produced by B. subtilis B6-1 with soybean and sweet potato residues as substrate, effectively suppressed cucumber wilts and significantly increased the dry weight of both roots and shoots. γ-PGA can also be used as a fertilizer synergist to increase winter wheat grain yield.

F. Gene Vectors and Vaccine Adjuvant

Biodegradable derivatives of poly L-Glutamic acid are suitable vectors for gene therapy. PGA derivatives reduce the surface charge and size of the DNA by forming polyelectrolyte complexes with DNA. Such complexes are able to protect DNA from digestion by DNase I and also facilitate the uptake of DNA by cells.

Several studies suggest that PGA can be used as a vaccine adjuvant. In one study, PGA used as adjuvant for recombinant Transmissible Gastro Enteritis virus nucleocapsid protein (TGEN) which was injected into rabbits. The group that received PGA with TGEN produced significantly more antibodies than the control group.

VI. CONCLUSION

Biopolymers have been a huge boon for mankind and an immediate answer for the polymer industry which focuses on environmental friendly applications and green chemistry. The Poly(γ-Glutamic acid) or Gamma Poly-Glutamic acid (γ-PGA) is an exciting biopolymer which has been recently in limelight as the compound and its derivatives have a broad spectrum of applications ranging from drug delivery to heavy metal absorbers. The high water retaining capacity of γ-PGA is enhanced by UV cross linking. Coupled with its non-toxicity, this makes γ-PGA very useful in environmental applications. PGA is the world's current obsession as a wonder amino polymer with lots of potential.

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