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Polyglutamic acid Applications in Pharmaceutical and Biomedical Industries

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ABSTRACT

Polyglutamic acid (PGA) is a natural biopolymer made up of periodical units of both L-glutamic acid, D-glutamic acid. This biopolymer is of special interest based on its biodegradability, non-dangerous and non-immunogenic properties, and thus it has been utilized effectively in many pharmaceutical applications. Among other novel applications, it has the likelihood to be utilized for protein crystallization, as a delicate tissue follower and a non-viral vector for safe quality conveyance. Therefore, PGA exhibited many therapeutic applications as anticancer agent, drug delivery, biological Glues, biological control agent, and in tissue Engineering. Investigations of PGA biosynthesis/production and learning of the catalysts and functional biological actions will additionally increase the range of its applications.

Keywords: polyglutamic acid, pharmaceuticals application, drug delivery, tissue engineering, anticancer,

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INTRODUCTION

Nowadays, biopolymers play very important role in our daily life based on their highly diversified applications. For many years, biopolymers are usually referred to carbohydrates polymers of microbial origin such as or proteins (polymer of amino acids). Cabohydrate biopolymers are mainly produced by microbes and find many applications in food, pharmaceutical and medical industries [1-3]. However, Polyglutamic acid (PGA) is a unique biodegradable, non-immunogenic and anionic homopolyamide biopolymer which made up of D- and L- glutamic acid units of wide range of molecular weight[4].Gamma PGAis a form where the peptide bonds are between the amino group of glutamic acid and the carboxyl group at the end of the glutamic acid side chain[5]. In general, PGA can be differentiated into 2 isoforms-poly-α-glutamic acid (α-PGA) and poly-γ-glutamic acid (γ-PGA) - depending on the attachment of the amino group to the carboxyl group (**Fig. 1**).

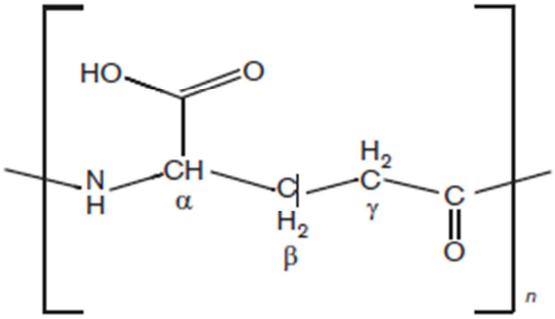

Fig. 1: Isoforms of Polyglutamic acid[6]

α-PGA is synthesized chemically by nucleophile initiated polymerization of the γ-protected N-carboxyanhydride of L-glutamic acid. Microbial production of α-PGA is difficult and the polymer can only be produced by recombinant microorganisms. γ- PGA has been produced extensively using bacteria, especially those of *Bacillus* sp. As PGA is different from proteins, because inside the cell, glutamate is polymerized via the γ-amide linkages, and thus synthesized in a ribosome independent manner [7].

Polyglutamic acid is a water-soluble, biodegradable polymer which produced by microbial fermentation. Recent research has shown that PGA can be used in drug delivery for the controlled release of paclitaxel [8]. By conjugating paclitaxel, or other drugs to PGA, the drug compounds become more stable and more water soluble. In addition, the conjugate can act as a drug depot for sustained release, enabling prolonged drug exposure to tumor cells [9]. Polyglutamic acid was first discovered by Bruckner (1937) when a capsule of *Bacillus anthracis* was released into the medium upon autoclaving [10]. Another naturally occurring source of Poly glutamic acid is the mucilage of natto (A traditional fermented soybeans in Japan), which contains a mixture of PGA and fraction produced by *Bacillus subtilis sawamura*[8]. Its widely known that, PGA is produced mostly by Gram positive bacteria, which include the genus *Bacillus*. However, it has also been reported that at least one Gram-negative bacterium (*Fusobacterium nucleatum*), some archaea and eukaryotes have the ability to produce c-PGA [12]. Polyglutamic acid has also been found in neurons of mice where it was covalently linked to tubulin [13]. Efforts have been made to insert the genes responsible for c-Polyglutamic acid production into *Escherichia coli* and in plants such as tobacco to increase the knowledge regarding the molecular mechanism of PGA production [14,15]. It was reported that pgsA, pgsB and pgsC genes were identified as essential for its production [16].In this review, we provide in brief a comprehensive update on the production; mechanism of synthesis, properties and new applications of bacterial PGA.

Mechanism and synthesis of Polyglutamic acid

Polyglutamic acid is water soluble, anionic, biodegradable and nontoxic homo-polyamino acid., and thus PGA and its derivatives are therefore interesting for broad range of industrial applications. However, one of the main challenges for PGA application is its high price as it is several tens to hundreds fold more expensive than the conventional materials it is envisioned to replace. Reducing the cost of production is the only foreseeable solution to this issue. Designing mass production systems would be a major step to introduce this biopolymer to the industrial society[17].To achieve this, one needs to study factors affect the yield of production. Information about genes and enzymes involved in PGA production would certainly help in manipulating organisms for more efficient production process[18].

The past 20 years have seen a rise in research in this direction and genes that play a role in every step of PGA production have been identified. This section attempts to provide an understanding of the current knowledge of the mechanism of PGA biosynthesis[19].

A biosynthetic pathway for the production of PGA has been proposed. L-Glutamic acid units that make up PGA can be derived from two sources. They can be obtained via the glutamic acid biosynthetic pathway either exogenously or endogenously. Endogenous production of L-glutamic acid requires conversion of a carbon source via acetyl-CoA and TCA cycle intermediates[20]. Exogenous L-glutamic acid can be converted to L-glutamine with the help of the enzyme glutamine synthesis. Figure: 2 explicate in detail the pathway process for the production of PGA.

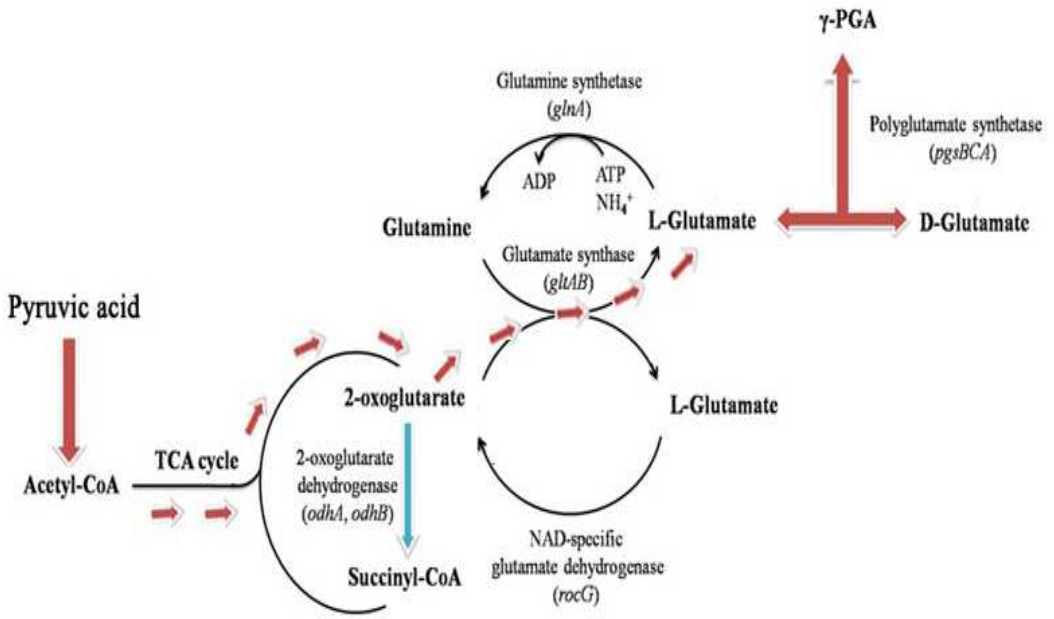

Figure 2: Biosynthetic pathway of polyglutamic acid[21]

The processes of PGA consist of four distinct stages: Poly glutamic acid racemization, Poly glutamic acid polymerization, Poly glutamic acid regulation and Poly glutamic acid degradation[22].

It have been also reported that PGA for man exocellular capsular polymer by many members of the genus *Bacillus*. From a research standpoint, *B. subtilis* and *B. licheniformis* are the most notable PGA can have molecular weights ranging from 100kDa to over 1,000kDa[23].However, the final molecular weight is dependent on many factors such as cultivation conditions and medium composition. However, cultivation time was considered as one of the main factors govern the molecular weight of the production PGA [24].

Table: 1Time Dependence of Poly glutamic acid Properties from *B. subtilis* IF03335[a][24]

Time (hr)	PGA g/l00 mL	Molecular Weight[b]			
		$10^{-3}M_n$	M_w / M_n	Polymer L- Isomer	Composition [c] D- Isomer
24	0	-	-	-	-
40	1.04	2951	3.8	19	81
47	0.84	4110	2.9	-	-
70	0.82	2610	2.8	20	80
94	0.66	266	10.5	-	-

a. The medium (100 mL) contained 3 g L-glutamic acid, 2 g citric acid,and 0.5 g ammonium sulphate. Cells were cultivated at pH 705 and 37 °C.
b. Measured by GPC.
c. Measured by liquid chromatography.

Production of PGA by microbial fermentation

Much research have been carried out for the production of PGA by bacterial fermentation. When PGA is produced synthetically, the product has low molecular mass of 10 kDa, which limits its application. Therefore, many research has done for the production of PGA using bacterial fermentation. However, the bacterial PGA can be as low as 10 kDa and sometimes reach higher molecular weight between 100 up to 1000kDa[25, 26]. The most common genus used for producing PGA is *Bacillus* and various strains such as *B. licheniformis* and *B. subtilis*. Bovarnick in 1942 that first showed that PGA is freely secreted into the medium after fermentation of *B. subtilis* [27].

Bacteria can produce PGA with different properties, such as molecular weight and enantiomeric composition [28]. Polyglutamic acid can also be produced in its perspicuous acid form or as a salt of the polymer in both of crystalline or amorphous forms[29].

These properties largely determine the application of PGA. The medium composition is one of the factors that can influence the properties of PGA and could be used as factor for biopolymer production of targeted characters and molecular weight[30].

However, PGA is generally diverse in its molecular structure, and during the fermentation it is co-produced with different polysaccharides and other biopolymers. Production of PGA from *B. subtilis* with a molecular mass of 26 kDa has proved to be challenging for various reasons: PGA synthesis or elongation is sometimes coupled with degradation of Polyglutamic acid towards the later stages of fermentation and the Poly glutamic acid synthesis complex itself is not stable[31].

However, *B. subtilis* cultivated in a medium with a high ammonium sulphate concentration has been found to produce super-high-molecular-weight PGA without the aforementioned problems. The high molecular weight PGA of average 26 kDa was obtained without the presence of any byproducts and was richer in L-glutamate than its D enantiomer. Also managed to isolate Poly glutamic acid which had a molecular mass .26 kDa, but it was difficult to accurately measure such a high-molecular weight polysaccharide. The high molecular weight PGA producing bacteria have been divided into two groups depending upon their nutrient requirement for PGA production – those that require L-glutamic acid in the medium and those that do not require L-glutamic acid. The L-glutamic-acid dependent bacteria include *B. subtilis*, *B. licheniformis* 9945. *subtilis* CGMCC 0833 [32],*B. subtilis* ATCC 15245 [33],*B. subtilis* C10 [34], *B. amyloliquefaciens* LL3 [35]. For L-glutamic- acid-dependent bacteria, the PGA yield increases with an increase in the L-glutamic acid concentration in the medium. **Table 2**: present some examples of PGA producer microorganisms.

Table 2: PGA production by different microorganisms

Organisms	applications	References
E. coli	*pgsBCA*genes that are responsible for γ-PGA production were cloned and expressed in *E. coli*. Low yields of γ-PGA were obtained (~0.024 g/l)	[36]
B. licheniformis S2	Newly isolated strain shown to have ability to reduce ammonium nitrogen content in swine manure by converting ammonium into biomass and γ-PGA.	[37]
B. subtilis Chungkookjang	Produces super-high-molecular-weight γ-PGA - 2×10^6 Da without any byproducts production.	[38]
B. subtilis CCTCC202048	Poly glutamic acid produced using solid state fermentations.	[39]
B. subtilis NX-2 &*Corynebacterium Glutamicum*	*Corynebacteriumglutamicum*have the ability to produce high levels of glutamic acid. Co-culturing bacteria for γ-PGA production eliminated the need to add exogenous L-glutamic acid and subsequently reduced the fermentation time and the production cost.	[40]
*B. mesentericus*MJMI	Bacteria isolated from Korean domestic bean paste. Poly glutamic acid consisted of 2000 glutamic acid residues.	[41]
B. licheniformis NCIM 2324	PGA acid produced using solid state fermentations. Maximum yield of 98.64 g g^{-1}.	[42]
B. licheniformis strain-R	PGA production by cells immobilized using an agar-alginate gel beads mixture (γ-PGA yield 36.75 g/l). Luffa pulp-adsorbed cells produced γ- Poly glutamic acid yield of 50.4 g/l.	[43]
Bacillus sp. RKY3	Newly isolated strain used for γ-PGA production. Maximum yield of 48.7 g/l was achieved using optimized medium in small scale bioreactor.	[44]

Different Applications of PGA

Biomedical and Pharmacological applications of Polyglutamic acid

Since its first discovery, PGA have been used in many pharmaceutical and medical applications. Medical uses of PGA have pulled in a considerable measure of enthusiasm for the most recent decade and much research has been completed here [45]. A portion of the more vital potential restorative uses of PGA are summarized in **Table 3**.

Table 3: Different applications of PGA in pharmaceutical and medical industries

Applications	Function	Reference
Drug delivery system	PGA with covalently attached cisplatin was shown to reduce the toxicity of cisplatin, whilst efficiently decreasing the tumor size of xenografted human breast tumors in nude mice as well as lengthen the survival of nude mice grafted with Bcap-37 tumor cells.	[46]
	Macromolecular conjugate of paclitaxel and L-PGA called paclitaxel poliglumex exhibited outstanding benefits over conventional paclitaxel. Paclitaxel poliglumex was accumulated in tumor tissue, where it gradually discharged the active agent paclitaxel.	[47]
Tissue engineering	PGA/chitosan composite biomaterial demonstrated potential application in tissue engineering as it is more hydrophilic and cytocompatible than conventional chitosan matrices.	[48]
	PEC of chitosan and PGA demonstrated potential application in wound dressing. The complex presented sufficient moisture content and showed good mechanical properties, which would allow the dressing to be easily Removed from the wound surface without destroying renewed tissues.	[49]
Biological glutinous	Mixture of gelatin and PGA aqueous solution which resulted in the formation of a hydrogel in the presence of water-soluble carbodiimide demonstrated better lung adhesion and air-leak sealing than conventional fibrin glue.	[50]
Calcium absorption	Administration of PGA increased calcium absorption in the intestine in post-menopausal women by inhibition of the formation of an insoluble calcium complex with phosphate. Can be potentially used for treatment of bone disorders.	[51]
Bitterness-sedative agent	PGA has been used as a bitterness-relieving agent	[52]
Antibacterial activity	Magnetite NPs coated with sodium and calcium salts of PGA demonstrated antibacterial activity against Salmonella enteritidis SEM 01 compared with the commercial antibiotics linezolid and cefaclor, and were cytocompatible. NaPGA-NPs were also active against E. coli ATCC 8739 and Staphylococcus aureus ATCC 10832, whilst CaPGA-NPs were active against *E. coli* O157 :H7 TWC 01.	[53]
Gene delivery	pDNA/polyethylenimine/ Poly glutamic acid complex was developed and used for gene delivery with very high transgene capability and low toxicity.	[54]
Inhibition of influenza virus	PGA- based glycopolymers (used to inhibit influenza virus) showed higher solubility in water and heat stability, and lower toxicity and immunogenicity, compared with glycopolymers without Poly glutamic acid.	[55]
Treatment of xerostomi	PGA promotes salivary secretion and produces a moisturizing effect. Use of c-PGA solved problems associated with xerostomi: difficulties in speaking, bad breath, dental caries, periodontal disease and mucosal infectious disease.	[56]
Moisturizer	Poly glutamic acid has been demonstrated to improve the qualities of skincare and haircare products, such as exfoliating, moisturizing and removing wrinkles.	[57]
Protein carrier	- Sustained delivery of bone morphogenetic proteins for bone regenerative therapy. - Potential vaccine carrier for systemic and mucosal administration. No cytotoxic effect. - Effective oral delivery of insulin. Intestinal adsorption of insulin was enhanced & prolonged reduction in blood glucose level was achieved.	[58] [59] [60]
Drug carrier	Yield of cis-dichlorodiammine platinum*(II)* (CDDP) incorporation into γ-PGA was 12.3% better than α-PGA. Higher antitumour activity than CDDP alone, while being less toxic than the free drug. Can potentially be used for treatment of breast cancer.	[61]
Monoclonal antibodies	No deactivation and increased stability of monoclonal antibodies when administered in γ-PGA nanoparticles.	[62]
Antitumourogenic agent	Oral administration of PGA induced Natural Killer (NK) cell-mediated antitumour immunity in mice bearing MHC class I-deficient tumors.	[63]
Biodegradable scaffold	PGA fibres cross-linked with cystamine improved growth of mouse L929 fibroblast cells. Can be used for biomedical and tissue engineering applications.	[64]
Biological antiadhesive	Cross-linked PGA reduced tissue adhesion over injured surfaces in a rat model by forming a viscous hydrogel over it.	[65]
Soft tissue adhesive	PGA/gelatin hydrogels used as tissue adhesive. The hydrogel was more stable than fibrin glue.	[66]
Wound healing	PGA complexes were shown to have good wound healing properties	[67]
Antibacterial activity	PGA-coated magnetite nanoparticles exhibited antibacterial activity against *Salmonella enteritidis*SE01.	[68]

1-Anticancer

It have been reported for many years biopolymers especially of those of carbohydrate polymers can anticancer agent through direct cytotoxic activity against cancer cell or through their activities as immune system stimulator [69-71].

As shown previously, PGA is made of two isomers D and L. The L- glutamic acid play major roles in biomedical applications as bio anticancer agent. L- glutamic acid a kelp fixing, recognized in 1908 by Japanese researchers in charge of improving flavor for nourishment is presently best referred to logically as monosodium glutamate [72]. Alternate names incorporate – S-(+) - GA, L-GA, 2-aminoglutaric acid, and an anionic type of MSG at

physiological pH known as glutamate. The vicinity of free type of glutamate, not connected to protein is said to improve flavor in nourishment. It is likewise called as sense of taste pleaser[73].

It is otherwise called levoglutamide, L-GA 5 amide, L-(+) - 2-aminoglutamic acid. It is incorporated to the body from glutamic acid and smelling salts in a vitality requiring response. Albeit unnecessary in wellbeing, L- glutamic acid is restrictively fundamental in anxiety and ailment[74].Concentrates on in the course of the most recent quite a long while have investigated the physiological part and restorative utility of these atoms in different infection conditions L-Glutamic acid assumes a vital part in the biosynthesis of purine and pyrimidine bases of DNA and RNA. It is metabolized to L-glutamine by L-glutamine amalgamation and this metabolic procedure is vital for typical upkeep of cells. Glutamine is a standout amongst the most copious amino acids and partakes in an assortment of physiological capacities, in particular - as a noteworthy fuel hotspot for enterocytes, as a substrate for gluconeogenesis in kidney, lymphocytes, and monocytes, a supplement/substrate in muscle protein digestion system in light of contamination, aggravation, and muscle injury. Thinks about assessing the part of glutamine have affirmed its support in keeping up mucosal uprightness of the gastrointestinal tract taking after its organization in patients with real inside surgery[75]. The part of glutamine as defensive operators in hepatobiliary brokenness and as a supplement altogether parenteral sustenance is settled, especially, in patients under escalated care. L-Glutamic acid physiologically exists as glutamate. Glutamate alongside glutamine assumes a noteworthy part in amino acid digestion system and in this way in keeping up nitrogen parity in the body[76].

2-Drug delivery system

PGA has been widely used in drug delivery platforms. This based on the presence of carboxyl groups on the side chains which offer correlation points for the coupling of chemotherapeutic agents. This make the drug more dissoluble and easy for controlled release application. It has also been use for vaccine encapsulation, immobilization and protein or adsorption for delivery system[77].The drug delivery market sector is right now encounter twofold digit development, with re venues of medication conveyance items anticipated that would surpass USD100 billion by 2020[78].Infuse capable, managed release innovations are the quickest development portion of the business sector. In this application, the medication atoms are attached to water dissolvable polymer, for example, poly glutamic acid by means of covalent holding, subsequently rendering the medication more solvent and less demanding to manage. On account of medication conveyance for hostile to growth operators, the polymer-drug conjugate enters the different specialists with a free hydroxyl bunch [79].

3-Biological Glues(gluten)

During lung and chest operations, air leakage is one of the main problem complicated by these leaks to shut down with the classic image technology such as sewing or stapling [80]. Some biological such as fibrin has been used for this application. Although fibrin, fibrinogen and thrombin are obtained from human blood transfusions and ensure the high biocompatibility, they are also a potential source of viral infection. Thus, some synthetic and semi-synthetic compounds, such as cyanoacrylate and urethane pre-polymers were applied to substitute the human origin compounds. However, these materials have different defects, toxicity comprehensive cells, and low degradation rate. Poly acid promise glue biodiversity as incurable quickly in the merger with the gelatin and water-soluble carbodiimide [81].

4-Tissue Engineering

Since the first start of tissue engineering as merging technology in 1992 [82],numerous experiments have been performed to differentiate biodegradable and biocompatible frameworks for recovering tissues and transportation stage of joined medications. A polymeric platform is noticeable not just to keep space and solidness for cell development, separation, and association, additionally to supply supplements. therefore, polylactic acid (PLA) or polyglutamic acid(PGA) are utilized for setting up the platform, however they have an impediment, for example, low usefulness, instigation of aggravation amid hydrolysis, and hardness confound to the encompassing tissues. As of late, utilization of PGA as a range has pulled in much consideration in tissue engineering sector [83].

5-Vaccine

PGA capsule is crucial for the virulence of *B. anthracis*, but interestingly this biopolymer capsule is non immunogenic. Thus, it's ensured to the protective matter (PA) of *B. anthracis* or completely different proteins to provide passive vaccines. Previous results have shown that PGA is that the simplest carrier, and thus the immunogenic conjugates containing Poly glutamic acid can elicit immune responses against *B. anthracis* and completely different bacilli [84].

While not acting as agent, PGA can be also applied as support matter transporter or immune adjuvant. Antigen carrying- PGA nanoparticles area unit proved to be eligible to causing active cellular and substance immune responses and should be useful as cogent immunizing agent adjuvants against infectious diseases [85]. For specific diseases, PGA nanoparticles sq. mensuration novel and safely adjuvants for Japanese redness immunizing agent [86]; a mixture of influenza hemagglutinin (HA) immunizing agent and amphiphilic γ-Poly glutamic acid -graft-lphenylalanine copolymers can induce raised immune reaction against influenza [87]. Moreover, hypodermic protection of mice with HIV-1 p24-encapsulating PGA nanoparticles can stimulate antigen-specific IFN-γ-producing T cells in spleen cells and induced p24-specific body fluid antibodies, whereas the nanoparticles play a vital perform in causing cellular immune responses. Thus, PGA nanoparticles encapsulating numerous antigens have nice potential as novel and economical protein-based vaccines [88, 89].

CONCLUSION

Polyglutamic acid is biodegradable, non-toxic, and non-immunogenic, and can therefore be used safely in a variety of applications. This important biopolymer has been exploited widely for medical applications, more so for sustained drug delivery. Poly glutamic acid can be obtained in large quantities without any chemical modification step. In addition, it is not susceptible to proteases and hence could provide better sustained delivery of conjugated drugs in the body. During last five years, the studies on the potential applications of PGA in drug delivery system, anticancer agents and in tissue engineering have resulted in a significant development in PGA production. The PGA composites need to be further improved to increase its market acceptability. However, its expected that in the next five years, more researches will be focused on the thorough appraisal for biodistrubutin, toxicity and pharmacokinetics before using PGA based materials in the clinical trials for cancer therapy. For example, the studies of PGA as vaccine candidates will more focus on developing the features to providing the delivery with suitable surface molecules for recognizing the immune system and much effective targeting. Its also believed that PGA will help in improving the treatment of many diseases and will play more significant roles in tissue engineering research. We hope that this review provides a comprehensive update for better understanding of the relevant concepts of PGA synthesis and applications, and provide some up to date information on the potential application of PGA in different sectors.

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