Biomedical Applications of Chemically and Microbiologically Synthesized Poly(Glutamic Acid) and Poly(Lysine)

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Abstract: This review article deals with the synthesis, physiochemical properties, and potential biomedical applications of two homo-poly amino acids. Poly-α-glutamic acid (α-PGA) and poly-α-lysine (α-PL) were synthesized by chemical synthesis. poly-γ-glutamic acid (γ-PGA) and poly-ε-lysine (ε-PL) were naturally occurring bio-materials that were produced by microbial fermentation. Poly(glutamic acid) (PGA) and poly(lysine) (PL) are water soluble, biodegradable, edible and nontoxic toward humans and the environment. As a result, they are suitable for various applications and have recently attracted considerable interest of the chemical industry. The distinguished features of PGA and PL also make them promising candidates for biomedical applications. The applications of PGA and PL in the areas of biomedical materials, drug delivery carriers and biological adhesives have been studied extensively and will be discussed in this review.

Keywords: Poly-α-glutamic acid; poly-α-lysine; poly-γ-glutamic acid; Poly-ε-lysine; Biosynthesis; Biomedical applications.

INTRODUCTION

Poly (amino acid)s are referred to a small group of polyamides that consist of only one type of amino acid linked by amide bonds. They are different from proteins that are polyamides composed of different kind of constituents. Poly (amino acid)s are important classes of biodegradable polymers which are currently being investigated and applied for a wide variety of surgical and pharmaceutical applications. Among them, poly(glutamic acid) (PGA) and poly(lysine) (PL) were studied most extensively.

There are two structurally different poly(glutamic acid) and poly(lysine), whose structures are depicted in Fig. (**1**). Poly-α-glutamic acid (α-PGA) and poly-α-lysine (α-PL) were poly (amino acid)s synthesized by chemical synthesis with amide linkage similar to that of proteins. They were made of L-glutamic acid and L-lysine connected by amide linkages between α-amino and α-carboxylic acid groups, respectively. Poly-γ-glutamic acid (γ-PGA) is an unusual anionic, naturally occurring homo-polyamide that is made of D- and L-glutamic acid units connected by amide linkages between α-amino and γ-carboxylic acid groups. In contrast, poly-ε-lysine (ε-PL) is an unusual cationic, naturally occurring homo-polyamide made of L-lysine connected between ε-amino and α-carboxyl groups. γ-PGA was first discovered by Ivánovics and co-workers [51] as a capsule *of Bacillus anthracis* which was released into the medium upon autoclaving or upon aging and autolysis of the cells. It is also well known that the mucilage of "natto" (fermented soybeans, a traditional food in Japan) is a mixture of poly(glutamic acid) and fructan produced by *Bacillus natto* [30]. Since Bovarnick [11] showed that γ-PGA was freely secreted into the growth medium of *B. subtilis* as a product on fermentation, several *Bacillus* species have been shown to produce γ-PGA outside the cells [2,15,19,38,41,47,50,61, 79,92,112,114]. ε-PL was accidentally discovered as an extra-cellular material produced by filamentous bacterium *Streptomyces albulus* ssp. lysinopolymerus strain 346 as a result of a screening for a Dragendorff's positive substances (i.e. alkaloids or quarternary nitrogen compounds) [102-104]. Although later a mutant of strain 346 was found to produce higher amount of ε-PL [43], no other bacterial strains or eukaryotes have so far been found to synthesize ε-PL.

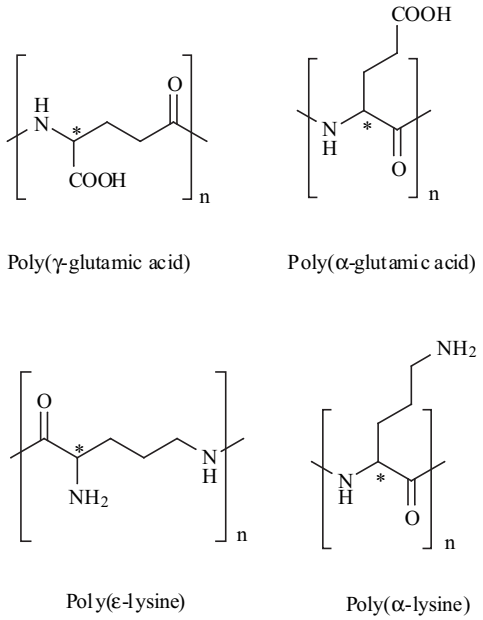

Fig. (1). Structure of poly(glutamic acid) and poly(lysine).

Poly(glutamic acid) and poly(lysine) are water soluble, biodegradable, edible and nontoxic toward humans and the environment. Therefore, potential applications of these bio-polymers and their derivatives have been of interest in the past few years in a broad range of industrial fields such as food, cosmetics, medicine and water-treatment [100]. There were a few mini reviews dealing with the biosynthesis, biodegradation and physico-chemistry of poly-γ-glutamic

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acid [47,80,100,115]. The multifarious applications of PGA (α- or γ-form) have been developed and reviewed by Shih and Van [100]. Thorough reviews on the synthesis and applications of PL (α- or ε-form) are scant. The aspects of the microbial biosynthesis and chemical modifications of ε-PL were briefly summarized by Kunioka [62] and others [84]. Since there is a revival in interest in using poly(glutamic acid), poly(lysine)and their derivatives as biomaterials for biomedical applications, much research has been done that has lead to a huge number of publications. The present review will be devoted to a survey of the main achievements in the chemical and microbial biosynthesis of these two bio-polymers. Furthermore, the applications of PGA and PL in the areas of biomedical materials, drug delivery carriers and biological adhesives will be extensively reviewed, on which much attention has been recently focused.

PRODUCTION OF POLY(GLUTAMIC ACID) AND POLY(LYSINE)

Chemical Synthesis of Poly-α-Glutamic Acid and Poly-α-Lysine

The chemical synthesis of poly-α-glutamic acid has had a long history. Nevertheless, continuous improvements have been made throughout the years. The most frequently used method for the preparation of high-molecular weight α-PGA is by nucleophile-initiated polymerization of the N-carboxyanhydride (NCA) of γ-protected-glutamate in appropriate solvents, which was followed by removal of the protecting group. The NCA of γ-protected-glutamate is readily prepared in a single step by refluxing γ-protected-glutamate with phosgene or trichloromethyl chloroformate in inert and dry solvents such as ethyl acetate or tetrahydrofuran (THF) [31,57]. In some instances solid triphosgen was used as cyclizing agent instead of phosgen gas, because it was more convenient to handle solid reagent than to handle toxic gas [72]. The most common protecting group for the γ-carboxylic acid is benzyl group which is readily removable by treatment of hydrogen bromide [49]. Piperonyl protecting group could be deprotected under milder condition using trifluoroacetic acid than hydrogen bromide [87]; therefore it was suggested to be a good substitute-protecting group for benzyl group. The ring-opening polymerization of the NCA intermediates were usually initiated by protic or aprotic nucleophile or base-initiators and carried out in aprotic solvents such as toluene, dioxane, chlorinated alkanes and DMF [8]. Primary amines were commonly used as protic initiators. On the other hand, aprotic initiators commonly used were tertiary amines or alkoxides. It is noteworthy that ring-opening polymerization by two types of initiators were suggested to be operating through different mechanisms as shown in Fig. (**2**).

The amine-initiated ring-opening polymerization of NCA generally led to α-PGA with a broad distribution of molecular weights, this is probably due to the diverse initiation and propagation steps involved. To achieve a narrower molecular-weight distribution, metal-catalyst initiators were thought. Polymerization of NCAs with nickel catalyst bipyNi(COD), where bipy is 2,2'-bipyridyl and

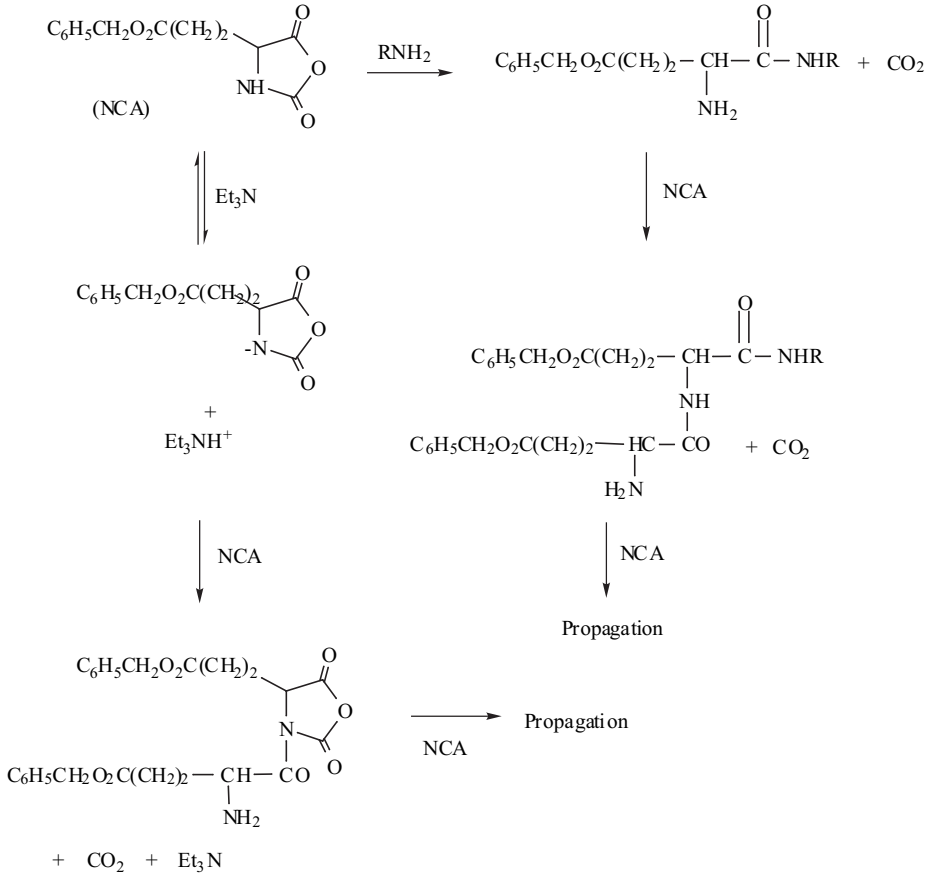

Fig. (2). Mechnism of the polymerization of NCA glutamate by nuceophile or base initiators.

COD is 1,5-cyclooctadiene, would lead to the minimization of chain transfer and chain termination reactions; hence resulted in polymers with narrower polydispersity (Mw/Mn=1.05-1.15; Mw and Mn are the weight- and number-average molecular weights, respectively) [22]. Impurities that interfere with initiators or NCA will inhibit the polymerization of NCA to high molecular product; therefore the removals of impurities such as chloride or moisture become critical in order to achieve high molecular polymers.

The preparation of poly-ε, N-carbobenzoxy-lysine was carried out by polymerization of monomer (ε, N-carbobenzoxy-α, N-carboxy-lysine anhydride) in bulk at a temperature of 105°C and a pressure of 10^{-4} mm Hg, or by polymerization of the anhydride in organic solvents using amines or sodium methoxide as initiators [5, 28, 55]. By varying the ratio of anhydride to initiator, polymers containing 5 to 240 amino acid residues per molecule were easily obtained. The carbobenzoxy protecting groups of poly-carbobenzoxy-lysine were removed by treatment with hydrogen bromide in glacial acetic acid [7].

Microbial Synthesis of Poly-γ-Glutamic Acid

As discussed earlier, several of *Bacillus* sp. produces γ-PGA as an extracellular viscous material or a capsular component. These strains are most useful in terms of industrial application and were studied most intensively. Work has been carried out on the nutritional requirements for cell growth, improving conditions for γ-PGA productivity and variation in chain D/L-repeat unit composition. In order to enhance the γ-PGA productivity, researchers have investigated the nutrient requirements for γ-PGA production and found that the nutrient requirements varied according to the strain used. According to the nutrient requirements, γ-PGA producing bacteria are divided into two groups; one requires the addition of L-glutamic acid to the medium to stimulate γ-PGA production and cell growth, the other does not require L-glutamic acid for γ-PGA production. The L-glutamic acid dependent bacteria most notably are *B. licheniformis* ATCC 9945A [112], *B. subtilis* IFO3335 [38], *B. subtilis* F-2-01 [61] and *B. subtilis (chungkookjang)* [2], and the L-glutamic acid independent bacteria most notably are *B. subtilis* 5E [79], *B. subtilis* TAM-4 [50] and *B. licheniformis* A35 [15]. Besides carbon and nitrogen sources, factors such as ionic strength, aeration, medium pH, all affected the productivity and quality of γ-PGA. Some poly-γ-glutamic acid producing bacteria are listed in Table 1, in which the nutrient requirement, cultivated conditions, productivity, and molecular weight are also summarized. The conditions for the production of γ-PGA by L-glutamic acid dependent and independent bacteria have been thoroughly reviewed by Shih and Van [100].

Medium E (L-glutamic acid 20g/l, citric acid 12g/l, glycerol 80g/l, NH_4Cl 7g/l, $MgSO_4 \cdot 7H_2O$ 0.5g/l, $FeCl_3 \cdot 6H_2O$ 0.04 g/l, K_2HPO_4 0.5 g/l, $CaCl_2 \cdot 2H_2O$ 0.15 g/l, and $MnSO_4 \cdot H_2O$ 0.04 g/l) is a medium mostly used to produce γ-PGA by L-glutamic acid dependent *Bacillus* species [20,66]. Extensive research has been done on the γ-PGA production by *B. licheniformis* ATCC9945A or *B. subtilis* IFO 3335 in such a medium or variations thereof [19,20,60,62,66,101,112,115,123]. The mechanistic pathways that lead to γ-PGA formation by strain IFO 3335 has been proposed and shown in Fig. (**3**). From the pathway, the authors proposed that γ-PGA formation comes largely from intracelluarly produced glutamic acid (from citric acid through isocitric acid and α-ketoglutaric acid in the TCA cycle) as well as extracellular glutamic acid.

Bacillus subtilis TAM-4 is an L-glutamic acid independent or *de novo* PGA producing bacterium. It does not have strain degeneration problems associated with some γ-PGA-producing strains [66,112,114]. It scarcely produced polysaccharides in medium containing glucose and it elongated with no change in the diastereoisomer ratio (78 : 22, D-isomer : L-isomer throughout the cultivation) in the molecule. These features distinguished this bacterium from

Table 1. Poly-γ-Glutamic Acid (γ-PGA) Producing Bacteria

Strains	Nutrients	Cultivated Conditions	Productivity (g/L)	Molecular Weight(*)	References
Bacillus Licheniformis ATCC 9945	Glutamic acid (20 g/L), Glycerol (80 g /L) Citric acid (12 g/L), NH_4Cl (7 g/L)	30°C, 4 days	17-23	1.4×10^5 - 9.8×10^5	[114, 20]
Bacillus subtilis IFO3335	Glutamic acid (30 g/L), Citric acid (20 g/L)	37°C, 2 days	10-20	1.0×10^5- 2.0×10^6	[62]
Bacillus subtilis TAM-4	Fructose (75 g/L), NH_4Cl (18 g/L)	30°C, 4 days	20	6.0×10^5 - 1.6×10^6	[50]
Bacillus licheniformis A35	Glucose (75 g/L), NH_4Cl (18 g/L)	30°C, 3-5 days	8-12	$3.0 \sim 5.0 \times 10^5$	[15]
Bacillus subtilis F02-1	Glutamic acid (70 g/L), glucose (1 g/L) Veal infusion broth (20g/L)	30°C, 2-3 days	50	1.20×10^6	[61]
Bacillus subtilis (natto)	Maltose (60 g/L), soy sauce (70 g/L) Sodium glutamate (30 g/L)	40°C, 3-4 days	35	---	[82]
Bacillus subtilis (chungkookjang)	2% glutamic acid, 5% sucrose, 2%($NH_4)_2SO_4$,	30°C, 5 days	13.5	10-1,000 kDa	[2]

*Molecular weight is dependent on culture conditions; --- Molecular weight is not determined

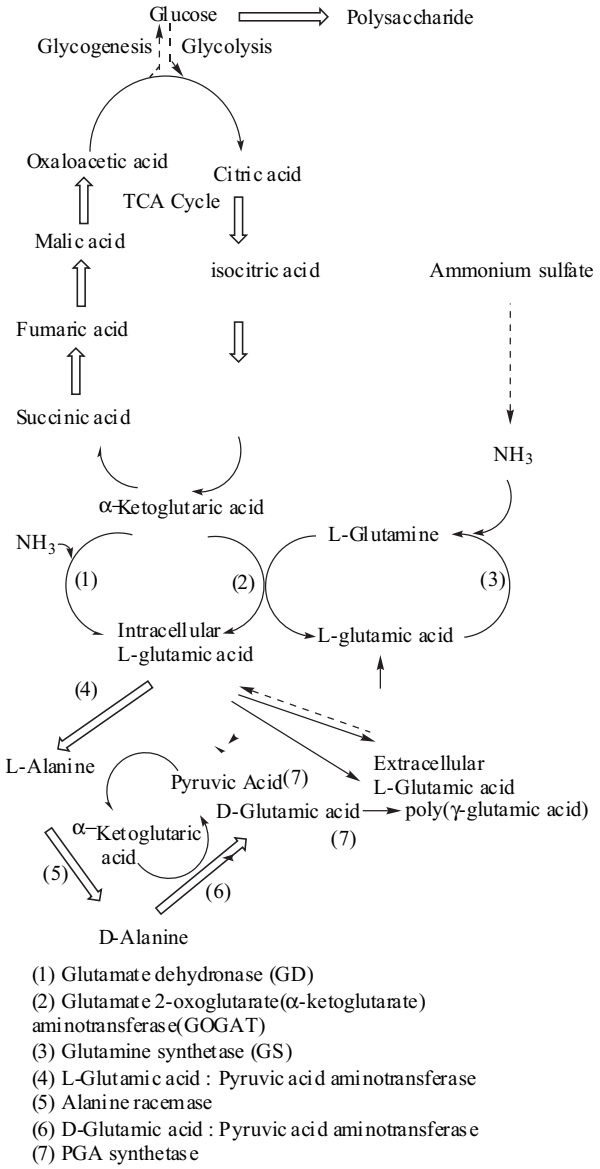

Fig. (3). Proposed pathway of γ-PGA synthesis in *B. subtilis* IFO 3335.

other γ-PGA-producing strains. In contrast to most of the bacteria produced γ-PGA under aerobic conditions, *B. licheniformis* strain A35 and strain S173 produced large amounts of γ-PGA under nitrate-respiration conditions [15,56]. Under optimized conditions, most of the bacteria produced γ-PGA in amounts ranging from 10-50 g/l in the culture medium, and the D/L glutamic acid content in the polymer was affected by the concentration of Mn^{+2} present in the medium [19,88]. According to the strain and culture conditions used for γ-PGA production, the weight-averaged molecular weight between 10^5 - $8x10^6$ and poly-dispersity between 2 -5 were typically reported.

Microbial Synthesis of Poly-ε-Lysine

As mentioned earlier, ε-PL was accidentally discovered as an extracellular material produced by filamentous bacterium *Streptomyces albulus* ssp. lysinopolymerus strain 346 more than 25 years ago [102-104]. Since then the production conditions have been investigated in the hope to optimize its production for commercial usage. Shima and Sakai reported that shake flask cultures of the wild strain of *S. albulus* grown in a basal medium (50g glycerol, 10g $(NH_4)_2SO_4$, 5 g yeast extract, 0.5g $MgSO_4$ 7 H_2O, 0.03g $FeSO_4$ 7 H_2O, 0.04g $ZnSO_4$ 7 H_2O in 1 liter of 1/50M KH_2PO_4-Na_2HPO_4 buffer (pH6.8)) typically yielded 0.3g/l of ε-PL after cultivation for 48 hr at 30°C. The typical fermentation process showed that the mycelial growth reached its maximum at 20hr of cultivation time. The subsequent decline in pH during the fermentation process was an essential conditions for the accumulation of ε-PL, which was produced after 30hr. To enhance the productivity, various approaches have been attempted. Shima and coworkers [105,106] carried out a two-step cultivation method. In such a method, *S. albulus* was grown in a mineral medium containing 20g/l glycerol and 5 g/l yeast extract for 1 day at 30°C. The cells were collected by filtration and inoculated into a mineral medium containing 20g/l glucose and 20 g/l citric acid and 10 g/l $(NH_4)_2SO_4$. A large amount of ε-PL, 4-5 g/l, could be produced in 8 or 9 days. Later Hiraki *et al.* [43] isolated a mutant of strain 346 by means of nitrosoguanidine treatment. The resulting S-(2-aminoethyl)-L-cysteine (AEC) plus glycine-resistant mutant produced four times higher amounts of ε-PL when the cells were grown in a new improved medium, M3G (50g/l glucose, 10g/l $(NH_4)_2SO_4$, 5 g/l yeast extract, 1.36g/l KH_2PO_4, 0.8g/l K_2HPO_4 , 0.5g/l $MgSO_4$ 7 H_2O, 0.03g/l $FeSO_4$ 7 H_2O, 0.04g/l $ZnSO_4$ 7 H_2O) at 30°C for 96h in reciprocal flask culture. It was found that the culture pH decreased from its initial value of 6.8 to 4.2 by 36h, and slowly decreased thereafter to 3.2 at 96 h. The accumulation of ε-PL in the broth increased significantly when pH was lower than 4.2. With the aim of enhancing the microbial production of ε-PL, a pH control strategy for cell growth and ε-PL production was employed by Kahar and coworkers [53]. The cultivation of *S. albulus* 410 was carried out in M3G medium in a 5-l jar fermentor by means of pH control strategy under extensive power consumption in a fed-batch culture. The pH control was divided into two phases. In phase I, cell growth was accelerated by maintaining the pH at higher than 5.0; in phase II, ε-PL production was increased by maintaining the pH at about 4.0. This control strategy successfully enhanced the production of ε-PL to 48.3 g/l form 5.7 g/l in the fed-batch culture. In a most recent study the possibility of the energy-saving production of ε-PL using *S. albulus* 410 in an airlift bioreactor (ABR) was evaluated, and compared with the production of ε-PL in a jar fermentor [54]. The results showed that the production level of ε-PL in a 5-l ABR with a power consumption of 0.3 kW/m^3 was similar to that in a 5-l jar fermentor with power consumption of 8.0 kW/m^3. Furthermore, the leakage of intracellular nucleic acid (INA)-related substances into the culture broth in the ABR was less than that in the jar fermentor, a fact that will minimize the difficulties in downstreaming processing on the recovery and purification of the polymer products. Apparently the use of ABR is promising for the low-cost production of ε-PL of high purity. The molecular weight of ε-PL was estimated by gel filtration on a Sephadex column. It mostly consists of only 25-30 constituents with a molecular mass approximately 4,000 and therefore has a much lower molecular weight than γ-PGA.

APPLICATION OF PGA IN MEDICINE

Drug Carrier

Poly(amino acid)s are of interest as drug delivery platforms because of their capacity to be both biocompatible as well as biodegradable to naturally-occurring biological products. Besides its biodegradability and biocompatibility, PGA (α- or γ-form) is also water soluble and nontoxic [84]. It has carboxyl groups on the side-chains that offer attachment points for the conjugation of chemotherapeutic agents, thereby rendering the drug more soluble and easier to administer. The α- or γ-PGA-drug conjugate can enters the tumor sites and the drug is released over time as the polymer biodegrades. A wide variety of anticancer agents have been conjugated to α- or γ-PGA and the resultant conjugates have been tested. The structures of these drugs are shown in Fig. (**4**) and the points of attachment to α- or γ-PGA are also shown in the structures. Table 2 summarizes various PGA- or PL-drug conjugates that have been synthesized and tested.

Paclitaxel (Taxol, TXL), a natural anti-microtubule agent extracted from the needles and bark of the Pacific yew tree (*Taxis brevifolia*), is a chemotherapeutic agent with potent antitumor activity against various human malignancies, including breast and ovarian tumors [46,91]. However, a major difficulty in the clinical use of paclitaxel has been its insolubility in water. To overcome the problem, TXL was conjugated to water soluble α- or γ-PGA *via* covalent bonding, thereby rendering it more soluble and easier to administer. The conjugate, PGA-TXL, exhibited markedly greater antitumor activity against murine tumors and human tumor xenografts than TXL [70,71]. Complete tumor regressions and cures were usually observed in both ovarine and breast cancer animal models when high doses of PGA-TXL was administered. Furthermore, this degree of antitumor activity can be achieved with single i.v. injection into animals. Preclinical data suggest that the uptake of PGA-TXL by tumor cells was ~ 5-fold greater than that of paclitaxel when equivalent doses were used. Once in the

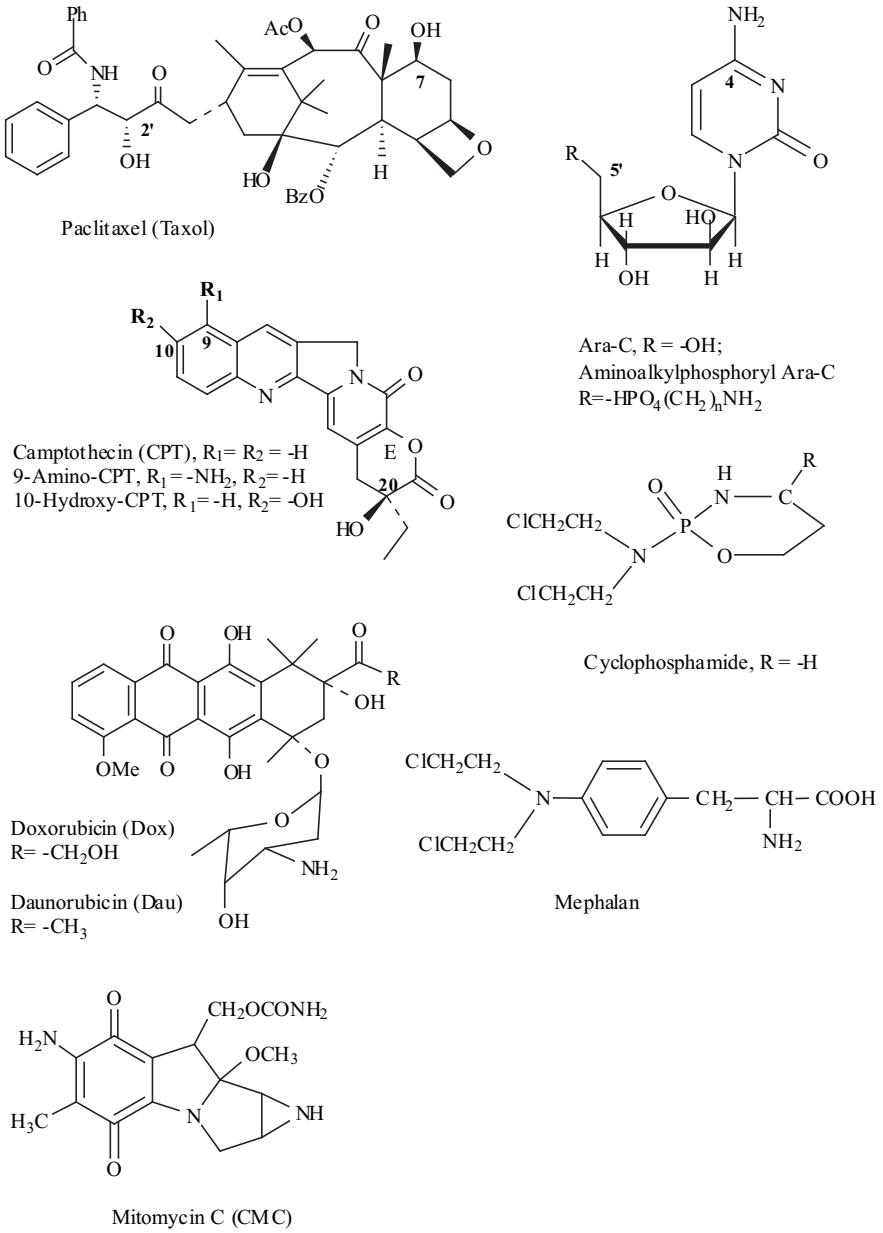

Fig. (4). Anticancer drugs that have been conjugated to PGA.

Table 2. Summary of PGA- or PL-Drug Conjugates Synthesized and Tested

Polymer	Drug	Activity	Ref
α- or γ-PGA[a]	Paclitaxel (TXL)	PGA-TXL showed complete tumor regression in both ovarine and breast cancer animal models (syngeneic tumors of xenografts inoculated s.c., i.p. or i.m. in rats or mice). Conjugate showed fewer side effects, markedly greater anti-tumor activity and higher maximum tolerated dosages (MTD) than free paclitaxel. In Phase I/II/III human clinical trials in the U.S and Europe.	[70,71,25,13]
α-PGA	Camptothecin (CPT)	PGA-CPT showed significantly enhanced anti-tumor activity in animal models of lung, colon and breast cancer, with up to 500% improvement over the free drug. Animal studies suggest that it permits up to 400% more drug to be administered without an increase in toxicity. In Phase II human clinical trials in the U.S.	[107,108]
α-PGA	Doxorubicin (Dox)	PGA-Dox showed less cytotoxic than free Dox to L1210 leukemia and B16 melanoma cells. *In vivo*, PGA-Dox is inactive, PGA-oligopeptides-Dox are active with anti-tumor activity increased with increasing length and degradability of the spacer.	[45,48,116,117,127]
α-PGA	Ara (C)[b]	Markedly less cytotoxic than free drug. *In vivo*, significa- ntly more efficacious than free drug to animal model (i.p. L1210 leukemia melanoma cells).	58,59]
α-PGA	Melphalan	Markedly less cytotoxic than free drug. *In vivo*, significa- ntly more efficacious than free drug to animal model (s.c. Yoshida sarcoma in rats)	[77]
α-PGA	Mitomycin C (MMC)	Markedly less cytotoxic than free drug. *In vivo*, less active than MMC in animal model (i.p. P388 mouse leukemia)	[90]
α-PL[c]	(poly I•poly C)[d]	The complex is 5 to 10 times more resistant to hydrolysis by ribonuclease of primate serum than the parent poly I•poly C. The complex induces significant levels of serum interferon in monkeys, chimpanzees and humans. In Phase II clinical trials in patients with recurrent anaplastic glioma.	[68, 14].
α-PL	Methotrexate (MTX)	The cellular uptake of conjugate was far greater (200-fold increase) than the uptake of free drug in cells that were either proficient or deficient in MTX transport. The conjugate markedly inhibited the growth of the drug resistant CHO PRO$^-$3 Mtx^{RII}5-3 cells.	[93-94,96-99]

[a] poly-α- or poly-γ-glutamic acid; [b] 1-β-D-arabinofuranosylcytosine; [c] poly-α-lysine; [d]double-stranded RNA polyriboinosinic–polyribocytidylic acid

cells, the polymer is digested, delivering a higher, more potent dose of paclitaxel directly to the tumor. Therefore, PGA-TXL is more powerful because of its unique ability to target the tumor. *In vitro* studies with PGA-TXL have clearly shown that the complex, in contrast to paclitaxel, supports neither tubulin polymerization nor the growth and survival of a Taxol-dependent CHO cell line [25]. Furthermore, PGA-TXL has prolonged half-life in plasma, and the extent and release of TXL in plasma is very low (< 0.1% in 144 h). From these results, PGA-TXL apparently showed fewer side effects and markedly improved anti-tumor activity, and allowed significantly increased maximum tolerated dosages (MTD) in animal model. PGA-TXL is currently in Phase I/II/III human clinical trials in the U.S and Europe [13].

Camptothecin (CPT) and its derivatives are important anticancer agents, it showed a broad spectrum of antitumor activities against many solid tumors in xenografts [35,36]. However, the lack of aqueous solubility and the instability of the pharmacologically critical E-ring lactone of the most CPT derivatives are two obstacles needed to be overcome for CPT derivatives to be clinical useful. Recently, a few water-soluble CPT derivatives were synthesized by coupling of CPT to α-PGA through the 20(S)-hydroxy group, with or without the use of a linker. These conjugates rendered reduced toxicity and improved efficacy *in vivo* [126]. For example, when H322 human lung tumor cells were inoculated intratracheally in nude mice, PGA-CPT administered in 4 injections at an equivalent CPT dose of 40 mg/kg per injection significantly prolonged the median survival of treated mice by four-fold compared with that of untreated control mice. In contrast, H322 tumor cells infected mice were not responsive to both CPT and cisplatin treatments. The LD_{10} of PGA-CPT after an i.v. single injection was 177 mg/kg of a CPT equivalent dose; in contrast, free CPT administered by the same manner resulted in toxic death at 40 mg/kg. The improved antitumor activity of the conjugate was attributed to several factors. These factors are: (1). The increased water-solubility makes the transportation of the drug conjugate to the tumor sites easier; (2). The enhanced permeability and retention effect of conjugate at abnormal tumor sites; (3). The graduate release of active CPT from conjugate prolonged contact between drug and tumor cells; (4). The well preservation by PGA of intact lactone-ring necessary for biological activity of CPT. Phase I clinical trials of PGA-CPT in patients with advanced cancers are currently underway in the U.S [13]. Recently, the effects of linkers between CPT and α-PGA, the point of attachment on the CPT and α-PGA molecules, and CPT loading on the polymer were evaluated to optimize PGA-CPT conjugate for best performance on the antitumor activity [107,108]. Results showed that PGA-Gly-CPT with an attachment on the 20(S)-hydroxyl group of CPT, a molecular weight of 49kDa and 37% loading (w/w) displayed best antitumor activity in nude mice with human colon and human lung carcinomas.

Besides TXL and CPT, PGA technology has been applied to a wide variety of other anticancer agents.

Doxorubicin (Dox) and other anthracycline have been conjugated to α-PGA, with or without chemical spacer, via amide, hydrolytically labile ester and hydrazones bonds [45,48,116,117,127]. These conjugates showed less cytotoxicity than their parent-unconjugated drug, a phenomenon observed for most PGA-drug conjugates. *In vivo*, the conjugates linked by enzymatically degradable spacers such as oligo-peptides were active with antitumor activity increased with the increase of oligopeptide length and degradation rate, whereas direct conjugates of α-PGA and Dox were completely inactive. As observed for the PGA-Gly-CPT conjugates, the anti-tumor activity of α-PGA and Dox conjugates increased with increasing molecular weight. Increasing the molecular weight of PGA-Dox conjugates from 14,000 to 60,000 at an equivalent Dox dose of 30 mg/kg resulted in enhanced antitumor activity, a result that was probably due to decreased renal clearance and increased plasma half-life of higher-molecular weight conjugate. Other anticancer drugs that have been conjugated to α-PGA included 1-ß-D-arabinofuranosylcytosine (Ara-C) [58,59], cyclophosphamide [4], L-phenylananine mustard (Melphalan) [77], mitomycin C (MMC) [90] and cis-dichlorodiammine- platinum (II) (CDDP) [3]. When α-PGA was attached to these cancer drugs, similar improvements in effectiveness and reduction in toxicity were observed.

Recently, a series of biodegradable derivatives of poly-L-glutamic acid, such as poly (γ-benzyl-L-glutamic acid), have been developed. These polymers showed great potential as drug delivery platforms [75] and suitable vectors for gene therapy [21]. Immunoconjugation of antibodies to PGA-drug conjugates has been developed for years to enhance targeted delivery of anticancer agents and to facilitate cellular uptake of the conjugate through specific tumor cell recognition [32,37,39,58,59,92,118,122]. The specific cellular binding followed by internalization rendered immunoconjugates selective cytotoxicity. Antitumor agents such as p-Phenylene diamine mustard, Mitomycin C, Doxorubin, Daunomycin were all successfully delivered to the target cells by the immunoconjugate technology. Techniques that ensure successful linkage between antibodies and PGA-drug conjugate have been developed. The linkages commonly seen were side-chain amide, side-chain thioether, side-chain hydrazone, terminal thioether, and terminal thioether via PEG (polyethylene glycol) spacer [72].

Biological Adhesives

Suturing has been the most common technique for tissue adhesion, control of massive bleeding, and wound closure in surgery. However, it is not very effective in hemostasis. In addition, it is not applicable to control continuous blood oozing from organ, seal air and body fluid leakage, or repair aortic dissections. In such cases, biological adhesives are commonly used. Synthetic and semi-synthetic surgical adhesives suffered from several drawbacks including cytotoxicity, low degradation rates, and chronic inflammation induced by the sustained release of their degradation products [113]. Currently, Fibrin glue [10,109] is the most widely used as surgical adhesive and hemostatic agent, but its adhesion property to tissues is poor, and mechanical strength is low. In addition, a risk of viral infection cannot be completely excluded because the fibrin glue is made from human blood. A new biological adhesive, formed by chemical crossing linking of gelatin and α-PGA, both of which are biodegradable, has been shown to be promising as a surgical adhesive and hemostatic agent that may possible replace the blood-originated fibrin glue [85,86]. The gelatin-PGA aqueous solution promptly forms a gel, when crosslinked with the aid of water-soluble carbodiimide (WSC), comparable to clinically used fibrin glues. The cured gel showed much higher bonding strength to soft tissue, and better hemostatic capability than fibrin. In addition the cured gel was slowly biodegraded in the body without inducing any problematic inflammatory response. More recently, a new potential biological adhesive made from porcine collagen and α-PGA has been developed, which is superior to fibrin in sealing air leakage from the lung [95]. Although most of the biological adhesives discussed above used synthetic α-PGA as a raw material, it is believed that natural occurring γ-PGA will be an ideal substitute [62,84].

APPLICATION OF PL IN MEDICINE

Due to its polycationic property, water solubility, biodegradability and biocompatabilty, PL (α- or ε-form) showed multifarious applications in the life science. In medicinal application, α-PL has been used to enhance efficacy of some interferon inducers, antiviral and antitumor agents [14,68]. It has also been shown to improve drug transport by reducing drug resistance, and to increase the efficiency of organelle fusion from hematocytes, liposome etc. Thus, many workers have investigated the use of α-PL in human drug-delivery systems, especially for gene delivery. Lately ε-PL has been applied in enzyme immobilization for the purpose of making enzyme sensors or enzyme reactors for clinical analysis [44,110].

The synthetic double-stranded RNA polyriboinosinic –polyribocytidylic acid (poly I•poly C) was effective as an endogenous interferon inducer [27]. Thus, it was supposed to be an effective antiviral and antitumor agent both therapeutically and prophylactically [33,67, 120,121,23,124]. Unfortunately, poly I•poly C was rapidly hydrolyzed by nucleolytic enzymes in primate (including man) sera, resulting in poor interferon induction. To overcame this setback, Levy and coworkers reported the formulation of a soluble complex, called poly (ICLC), which was prepared by complexing of poly I•poly C and α-PL in 0.5% carboxymethyl cellulose (CMC). The poly (ICLC) complex was 5 to 10 times more resistant to hydrolysis by ribonuclease of primate serum than the parent poly I•poly C. In addition, the poly (ICLC) complex induced significant levels of serum interferon in monkeys, chimpanzees and humans under conditions in which poly I•poly C itself induced no interferon [68, 14]. This primate-effective interferon inducer is now being used in Phase II clinical trials in patients with recurrent anaplastic glioma [26,64,69,81]. No toxic effects due to poly (ICLC) complexes were observed.

The antifolate agent methotrexate (MTX) is one of the most widely used drugs in the treatment of human leukemia, sarcomas, and other forms of neoplastic diseases [12]. However, resistance toward methotrexate has been

encountered in many instances due to deficient MTX transport. α-PL was shown to be avidly taken up by cultured cells and was used as carrier for MTX. Conjugation of MTX to α-PL markedly increased its cellular uptake and offers a new way to overcome drug resistance related to deficient transport. Sheng and coworkers [93-94,96-99] have conjugated MTX to a poly-α-(D-lysine) of 60,000 Mr fragment and to α-PL of molecular weights varying from 3,100 to 130,000 and measured their cellular uptake and growth inhibitory effects on 3 lines of Chinese hamster ovary (CHO) cells. The cellular uptake of conjugate was far greater (200-fold increase) than the uptake of free drug in cells that were either proficient or deficient in MTX transport. Both L- and D-isomeric conjugates were taken up by cells in a comparable fashion. The L-isomeric conjugate markedly inhibited the growth of CHO PRO^{-}3 Mtx^{RII}5-3 cells, a mutant line known to be drug resistant because of deficient MTX transport. In contrast D-isomeric conjugate showed no effect on either resistant or normal CHO cells. It was further shown that the strong growth inhibitory effect of the L-isomeric conjugate was due to the intracellular hydrolysis of the conjugate with subsequent liberation inside the cell of a small pharmacologically active MTX adduct. A such adduct was detected in the cells exposed to the L-isomeric conjugate, but undetectable in cells exposed to the D-conjugate. Apparently the lack of inhibitory effect of the D-isomeric conjugate was not due to less cellular uptake but was due to lack of breakdown of the poly-α-(D-lysine) carrier. Data also showed that in methotrexate-resistant cells the rate of intracellular release of active drug after uptake of conjugate was the same order of magnitude as the rate of uptake of the free drug by transport-proficient cells. Hence, PL-mediated transport of a drug can overcome drug resistance due to deficient transport. The above studies demonstrated that α-PL was a potentially versatile drug carrier because of the following reasons: (1). it is easily uptaked into the cells; (2). it carries many amino functional amino groups to which drug can be covalently attached; (3). it is available in a broad range of molecular sizes that may be tailored to specific needs; (4). it is readily degraded and nontoxic in the target cells. Furthermore, a drug covalently conjugated to α- PL carrier can be released inside cells a pharmacologically active adduct that is more effective than the free drug per se.

Since Friedmann outlined prospects for human gene therapy [29], the use of genetic materials (i.e. genes, antisense oligonucleotides, ribozymes, and triple-helix forming nucleotides) as therapeutic agents for modification of somatic cellular genotype has shown rapid progress in the treatment of a broad spectrum of diseases [40, 78]. A major technical impediment to introduce genes to the cells is the lack of ideal gene delivery systems. To date a number of techniques have been developed for the introduction of genes into mammalian-cultured cells, but their efficiency of transfection *in vitro* is not reproduced *in vivo*. Although the use of retroviral or adenoviral vectors [34,89] for transfection *in vivo* has achieved some, albeit limited, success, it still suffers several disadvantages. For example, adenoviral vectors have high immunogenicity, which restricts the repeated use of the delivery system, and retroviral vectors have potential viral-associated toxicity, including viral replication via endogenous virus recombination and oncogenic effects via insertional mutagenesis [52,111]. For these reasons, nonviral vectors composed of non-immunogenic self-assembling components are attractive alternatives to viral systems for gene therapy. The impetus to develop nonviral gene delivery vectors has led to examination of cationic polymer-based gene delivery vectors [125]. Cationic polymers have been shown to form complex (polyplex) with plasmid DNA via ionic interactions, thus protect DNA from nuclease degradation and serve as platform for enhancing the cellular delivery of DNA [16,24]. It is well known that α-PL strongly binds to DNA to induce compaction of the DNA molecule [63]. However, the stoichiometric complex consisting of α-PL and DNA suffers from drawbacks (e.g. low solubility due to charge neutralization, cytotoxicity and low transfection efficiency) that limited its clinical use as *in vivo* gene carrier [6,65,119]. As a result, α-PL modified with various substances to control and optimize complex properties has been established. For example, poly(ethylene glycol) (PEG)-grafted PL (PEG-g-PL), or A-B type block polymer with one hydrophilic polymer (PEG) region combined with one polycationic polymer (α-PL) region, was synthesized and shown to make a complex with plasmid DNA, resulting in lower cytotoxicity and improved transfection efficiency and cell viability in comparison with α-PL alone [17]. Recently, lactose was attached to PEG-PL block polymer and formed a Lac-PEG-PL carrier for targeting hepatoma cells specifically [18]. The new Lac-PEG-PL carrier can form a complex with plasmid DNA and serve as an efficient gene delivery carrier with higher solubility and lower toxicity compared to that of α-PL. Many other modifications of α-PL for specific cell targeting and better gene delivery *in vivo* have been published in the literature [1,42,76]. Besides gene delivery, the modified PEG-PL copolymer has also been used effectively as a carrier for cis-diaminedichloroplatinum (cisplatin, cDDP), an antineoplastic agent widely used in the treatment of teticular, ovarian, head and neck tumors [9].

Other applications of PL or its derivatives in the biomedical fields included preparation of ε-PL adsorbents and use for selective removal of endotoxins (lipopolysaccharides; LPS) from cell products used as drugs [44], immobilization of glucose oxidase on PL-modified polycarbonate membrane to be used as a glucose sensor [110]. Recently, micro or nano-capsules containing α-PL or its derivatives have been designed for delivery of ocular drug or encapsulation of cell lines useful for the delivery of bioactive molecules *in vivo*. Because these capsules are semi-permeable and biocompatible, the encapsulated cells could remain vital and secrete the desired therapeutic agents either continuously or in response to specific physiological stimuli in targeted recipients. For example, SK2 hybridoma cells microencapsulated in an alginate-PL-alginate (APA) membrane (APA-SK2 cells) produced and secreted anti-human interleukin 6 (hIL-6)monoclonal antibodies (SK2 mAb), which was very effective in therapy of IgG1 plasmacytosis and mesangio-proliferative glomerulonephritis in hIL-6 transgenic mice [83]. The APA microencapsulated Leydig cells, when delivered intraperitoneally into castrated rats, secreted testosterone and maintained it at a level of 0.51 ng/ml for more than three months without human chorionic gondotropin stimulation [73]. The encapsulation of ovarian cells for the secretion of progesterone and estrogen in culture

and *in vivo* has been demonstrated through the use of APA micro-encapsulation technique [74].

CONCLUSION

PL (α- or ε-form) and PGA (α- or γ-form) have been known for years and numerous researches have been carried out on these polymers. The chemical synthesis of α- PL and α-PGA was well established. In contrast, the microbial synthesis of ε-PL and γ-PGA was more elusive, because the mechanism and even the principle substrate involved in the polymer formation are still not fully understood, and serious conflicts often exist within the literature. Although commercial production of PGA (α- or γ-form) or PL (α- or ε-form) *via* chemical or microbial methods has been achieved, the yields of polymers via microbial synthesis still need to be continually improved. Increased knowledge of the biosynthetic mechanisms will allow the design of γ-PGA or ε-PL overproducers which are genetically and metabolically modified in the mass production of polymers and in the ability to secret polymer effectively. In addition, future works also need to be focused on custom design of polymers that were tailor-made to control product structure (e. g. varied stereochemical composition or molecular sizes) and function (e. g. varied biodegradability or water-solubility). The elaboration of either biotechnological or technical procedures for the production of polymers of diverse structures to meet special demand of practical application are being launched and will soon provide a broad spectrum of new PGA and PL.

PGA (α- or γ-form), PL (α- or ε-form) and their derivatives have proven to be effective as drug carriers. The water-soluble polymer thereby rendered the drug more stable, soluble and easier to administer. In addition, the conjugate can act as a drug depot for sustained release, enabling prolonged drug exposure to target cells. Also by targeting, systemic toxicity is reduced. Some of the conjugates have entering clinical trials, and the results are promising. Future emphasis will focus on the synthesis and characterization of novel PGA or PL-based polymers to improve their pharmacological properties and on the development of targeted drug delivery systems. In addition to drug delivery platform, PGA and PL polymers and their derivatives have been suggested for many other biomedical applications. Applications in areas such as biological glues, medical wound dressing, medical suture, cells encapsulation, bio(chemical) sensing have been explored and significant results have been achieved. In order to make the commercial applications of PGA and PL in biomedical fields viable, much works still need to be done. Nonetheless, the technological contents described above provide an interesting starting basis for further development of these two environmentally friendly poly(amino acid)s in biotechnological applications.

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