

Award Review
Occurrence, Biosynthesis, Biodegradation, and Industrial and Medical Applications of a Naturally Occurring ε-Poly-L-lysine

Yoshimitsu HAMANO
Department of Bioscience, Fukui Prefectural University,
4-1-1 Matsuoka-Kenjojima, Eiheiji-cho, Yoshida-gun, Fukui 910-1195, Japan

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ε-Poly-L-lysine (ε-PL) consists of 25–35 L-lysine residues with linkages between the α-carboxyl groups and the ε-amino groups. It exhibits antimicrobial activity against a spectrum of microorganisms, including bacteria and fungi. Because of its high levels of safety and biodegradability, it is used as a food preservative in several countries. We recently identified an ε-PL synthetase (Pls) as a membrane protein, and investigated the catalytic mechanism. Pls was found to be an unusual non-ribosomal peptide synthetase (NRPS)-like peptide synthetase producing ε-PL synthetase (Pls) as a membrane protein, and investigated the catalytic mechanism. Pls was found to be an unusual non-ribosomal peptide synthetase (NRPS)-like peptide synthetase producing ε-PL with chain-length diversity. In addition, transcriptional analysis of pls and a kinetic study of Pls further suggested that the Pls catalytic function is regulated by intracellular ATP, high levels of which are required for full enzymatic activity. Furthermore, it was found that acidic pH conditions during ε-PL fermentation are necessary for the accumulation of intracellular ATP, rather than inhibition of the ε-PL-degrading enzyme.

Key words: ε-poly-L-lysine; biopolymer; food preservative; non-ribosomal peptide synthetase; membrane protein

Biopolymers are the most abundant molecules in living matter. Microorganisms are capable of producing a wide variety of biopolymers, including polynucleotides, polyamides (proteins), polysaccharides, polyesters, and polyketides. However, homopolymers, which are made up of only a single type of amino acid, are far less ubiquitous. In fact, only two amino-acid homopolymers are known to occur in nature: γ-poly-glutamic acid (γ-PGA) and ε-poly-L-lysine (ε-PL).1) The latter, which consists of 25–35 L-lysine residues with linkages between α-carboxyl groups and ε-amino groups (Fig. 1), is produced by Streptomyces albulus No. 346, now designated S. albulus NBRC 14147.1–6) The compound is biodegradable and watersoluble, and has various functions including antimicrobial activity,5) antiphage action,5) endotoxin-selective removal action,7) and antiobesity action due to inhibition of pancreatic lipase.8) Since this polymer has been practically non-toxic in acute, subchronic and chronic feeding studies in rats,9) and non-mutagenic in bacterial reversion assays,10) it is widely used as a natural food preservative in certain countries, including Japan, Korea, and the United States. The antimicrobial activity of ε-PL is dependent on its molecular size.11) Shima and coworkers found that ε-PL, with more than nine L-lysine residues, severely inhibited microbial growth, but the L-lysine octamer demonstrated negligible antimicrobial activity.11) In contrast, chemically synthesized α-poly-L-lysine, which contains a considerably longer chain of L-lysine residues (50 residues) that show linkages between the α-carboxyl and ε-amino groups, demonstrates a lower activity than ε-PL. Hence, polymerization of L-lysine via an isopeptide bond is required for its biological activity, and the polymerization mechanisms involved in the chain-length diversity of ε-PL are of particular interest.

This review focuses on current knowledge and the most recent advances in regard to the occurrence, biosynthetic mechanisms, biodegradations, and industrial and medical applications of ε-PL.

I. Occurrence of ε-PL in Microorganisms

This characteristic polymer was discovered as a Dragendorff-positive substance produced by actinomycete S. albulus NBRC14147 from the soil.2–4) Since the discovery of the S. albulus strain, no microorganisms producing ε-PL were isolated until recently, when a novel screening method succeeded in isolating several strains of Streptomyctaceae and ergot fungi.12) This method detected basic polymers such as ε-PL, that interact with a charged dye embedded in agar plate. From 300 soil samples, more than 10 ε-PL-producing strains were found by this simple method. The ε-PL-producers obtained were identified as member of the genera Streptomycyes, Streptoverticillum, Kitasatospora, and Epichloë. Kitasatospora kifunense strain MN-1 and Epichloë sp. MN-9 produced ε-PL with short chain-lengths (~29 mer).

Hirohara and coworkers have reported that the two-stage culture method was effective for the screening of microorganisms producing ε-PL.13) At the first stage, a strain was inoculated into a test tube containing a growth culture medium and incubated for 20–48 h at pH 6.8 and
30 °C (cell growth culture). At the second stage, the mycelia collected by centrifugation were resuspended in production medium and cultured for up to 7 d at pH 4.5 and 30 °C (ε-PL production culture). Of the 1,900 actinomycete colonies isolated from soil samples, about 50 strains were found to produce large amounts of ε-PL. Among these 50 strains, 10 strains along with the Streptomyces genus, and the chain-length of the ε-PLs produced were studied in detail. All 10 strains were identified as the Streptomyces genus, and the domain architecture of Pls and schematic catalytic mechanisms are shown (a). Gene organization around the pls gene (accession no. AB385841) is shown (b). Small arrows indicate the PCR primers used in this study.

**II. Identification of an ε-PL Synthetase, Pls**

Non-ribosomal peptide synthetases (NRPSs) are multifunctional enzymes consisting of semiautonomous domains that synthesize a myriad of secondary metabolites. Using an assembly-line logic comprising multiple modules, these enzymes utilize a thiotemplated mechanism to activate, tether, and modify amino-acid monomers, sequentially elongating the peptide chain and finally releasing the complete peptide. The order and number of modules of an NRPS system determine the polymers using 2,4-dinitrophenol (DNP) indicated that the polymer consists of 14 residues. Mass spectra identical to the reference standard polymer with 14 residues gave results identical to that of the reference standard polymer with 14 residues. Mass spectra identical to the reference standards were also observed for products of other chain lengths. Chemical modification of the synthesized polymers using 2,4-dinitrophenol (DNP) indicated that the α-amino groups of the ε-lysine residues were labeled with DNP. This result indicates that the polymer consists of ε-lysine residues with linkages between the α-carboxyl and ε-amino groups, and confirms that the polydisperse polymer products are indeed ε-PL.

Yamanaka et al. investigated other enzymatic properties. The enzyme required Mg²⁺, ATP, 20–30% glycerol, 2 mM dithiothreitol, and 0.2–0.4% NP-40 for full activity. ATP was converted to AMP during the Pls reaction. No activity was detected with other nucleotides, such as GTP, CTP, and TTP, in the Pls polymerization reaction. Maximum activity occurred at an optimum pH of 8.5. The effect of temperature on the enzyme activity was investigated over a range of 10–45 °C, the maximum activity being observed at 25–30 °C.

**III. Catalytic Mechanism of Pls**

Based on the amino acid sequences of Pls, the pls gene was identified. A BLAST database search indicated that homologous genes are widely distributed among microorganisms. The pls gene encoded a protein of 1,319 amino acids, containing the internal amino-acid sequences previously determined. The calculated molecular mass (138,385 Da) was in good agreement with the results of the SDS–PAGE analysis of the purified Pls.

In a traditional NRPS, the amino-acid substrate is activated as an acyl-O-AMP by an adenylation domain (A-domain), and is subsequently loaded onto the 4'-phosphopantetheine (4'PP) arm of the adjacent thiolation domain (T-domain), resulting in the formation of an acylthioester and AMP release. A domain search indicated the presence of an A-domain and a T-domain in the N-terminal region of Pls (Fig. 3). Surprisingly, Pls had no domain with significant sequence similarity to the traditional condensation domains (C-domains), which are crucial in peptide bond formation in NRPSs. Furthermore, it had no traditional thioesterase domain (TE-domain), the domain that catalyzes the release of synthesis was observed in an insoluble fraction, suggesting that Pls is an insoluble protein such as a membrane protein. Yamanaka et al. therefore solubilized this fraction with a non-ionic detergent, Nonidet P-40 (NP-40). By successive purification steps including column chromatography, the solubilized Pls was finally purified 168-fold to apparent homogeneity. The relative molecular mass of the native enzyme as estimated by gel-filtration chromatography was 270 kDa, but the molecular mass as estimated by denaturing SDS-polyacrylamide gel electrophoresis (SDS–PAGE) was 130 kDa, suggesting that Pls is a homodimer.

The purified enzyme was incubated with ε-lysine and ATP. A polydisperse group of enzyme-dependent polymer products were detected, with their retention times, corresponding to those of reference standard polymers consisting of 3–17 residues (Fig. 2). ESI-tandem MS (ESI-MS/MS) analysis of the enzymatically-synthesized polymer with 14 residues gave results identical to that of the reference standard polymer with 14 residues. Mass spectra identical to the reference standards were also observed for products of other chain lengths. Chemical modification of the synthesized polymers using 2,4-dinitrophenol (DNP) indicated that the α-amino groups of the ε-lysine residues were labeled with DNP. This result indicates that the polymer consists of ε-lysine residues with linkages between the α-carboxyl and ε-amino groups, and confirms that the polydisperse polymer products are indeed ε-PL.
the final product from NRPS enzymes by hydrolysis to the free acid or cyclization to an amide or ester. Instead, a physicochemical analysis of the Pls amino-acid sequence with SOSUI\(^{19}\) suggested the existence of six transmembrane domains (TM-domains) surrounding three tandem soluble domains that displayed significant sequence similarity (with pairwise identities of 27%, 22% and 23%). Alignment of the tandem domains with traditional C-domains indicated that the tandem domains contained motifs showing similarity to His-motifs, or the HHxxxDG sequences found in all traditional C-domains, but the two histidine residues, known to be critical to catalysis, were not conserved. However, both the primary sequence and the predicted three-dimensional structure of these domains showed similarity to acetyltransferases, which show structural similarity to C-domains.\(^{20,21}\) For this reason, Yamanaka \textit{et al.} named the tandem sequences the C1-, C2-, and C3-domains, with the expectation that they would have a role in peptide bond formation.\(^{18}\)

Yamanaka \textit{et al.} explored the catalytic mechanism of Pls by ATP–PP\(_i\) exchange assay.\(^{19}\) They observed Pls-mediated adenylation of \(\text{l-lysine}\), but not any other proteinogenic amino acid. Using a His-tagged recombinant Pls containing only the A-domain and the T-domain (rPls-AT), the substrate \(\text{l-lysine}\) was found to be covalently attached to the enzyme. In addition, it was found that the three tandem domains (the C1-domain, C2-domain, and C3-domain) of Pls are essential for catalyzing the \(\text{l-lysine}\) polymerization reaction. To gain a better understanding of the function of the tandem domains, two additional recombinant Pls enzymes, rPls-ATC1C2 (lacking the C3-domain) and rPls-ATC1 (lacking both the C2-domain and the C3-domain), were constructed. As observed for rPls-AT, which lacks all three C domains, no polymer products were detected for rPls-ATC1C2 or rPls-ATC1, suggesting that either the C3-domain or the interconnected action of all three domains is essential for peptide-bond formation.

\begin{figure}[ht]
\centering
\includegraphics[width=\textwidth]{fig2}
\caption{Identification of Pls Reaction Products.}
\end{figure}

The reaction mixture (lower chromatogram and ESI mass spectrum) and a hydrolysate of \(\varepsilon\)-PL (upper chromatogram and ESI mass spectrum) were analyzed by HPLC/ESI-MS. The \(\varepsilon\)-PL hydrolysates (0.1 mg/mL) were prepared by hydrolysis with 1 N HCl.

\begin{figure}[ht]
\centering
\includegraphics[width=\textwidth]{fig3}
\caption{Domain Architecture of Pls and Schematic Catalytic Mechanisms.}
\end{figure}

The A-domain, T-domain, six transmembrane (TM)-domains, and three tandem domains (the C1-domain, C2-domain, and C3-domain) are shown schematically. The numbers on Pls are amino-acid residue numbers. The tandem domains iteratively catalyse \(\text{l-lysine}\) polymerization with free \(\text{l-lysine}\) polymer (or monomer in the initial reaction) as the acceptor and Pls-bound \(\text{l-lysine}\) as the donor, directly yielding \(\varepsilon\)-PL chains of diverse lengths.
Pls is predicted not to have a TE-domain, which is traditionally required for release of the product from the NRPS machinery. This suggests that the growing polymer products are not covalently attached to Pls during the polymerization reaction. To investigate this hypothesis, Yamanaka et al. employed L-lysine ethyl and methyl esters as substrates. HPLC/ESI-MS analysis of the reaction revealed that Pls produced the corresponding ethyl and methyl ester forms of ε-PL. While retention of final ester functionality confirmed that the growing polymers and polymer products are not tethered to Pls via covalent bonds such as thioesters or esters during the polymerization reaction (which would result in complete loss of the ester group), it also raised the question how the enzyme catalyzes polymerization using ester substrates at all. The answer lay in a unique function of Pls: the A-domain converts these L-lysine esters to L-lysine by its own esterase activity, and then the resulting L-lysine is adenylated and loaded to the T-domain.

In sum, Pls is a membrane protein with an A-domain and a T-domain characteristic of NRPSs. Although the order and number of modules of the traditional NRPS system determine the sequence and length of the peptide product respectively, Pls, an unusual NRPS-like peptide synthetase, produces ε-PL with chain-length diversity (25 mer to 35 mer, Fig. 3). The catalytic mechanism is initiated at the N-terminus by the A- and T-domains with adenylation and the transfer of an incoming L-lysine monomer as an extending unit. The C-terminal tandem domains (the C1-, C2-, and C3-domains) catalyze peptide bond formation between the extending unit and a freely diffusible L-lysine molecule (priming units), producing an L-lysine dimer, although the individual functions of the three tandem domains remain unclear. The dimer is then used as a freely diffusible substrate (acceptor substrate) for the next polymerization reaction. Since this catalytic cycle has no predetermined endpoint, Pls acts iteratively for ε-PL. While retention of final ester functionality confirmed that the growing polymers and polymer products are not tethered to Pls via covalent bonds such as thioesters or esters during the polymerization reaction (which would result in complete loss of the ester group), it also raised the question how the enzyme catalyzes polymerization using ester substrates at all. The answer lay in a unique function of Pls: the A-domain converts these L-lysine esters to L-lysine by its own esterase activity, and then the resulting L-lysine is adenylated and loaded to the T-domain.

IV. ε-PL Degradation

In vitro, Pls produced ε-PL with different chain lengths, ranging from three to 17 residues, indicating that the chain-length diversity of ε-PL is directly generated by the synthetase rather than via differential degradation of a uniform polymer by ε-PL-degrading enzymes.\(^{18}\) However, in vivo, it was unclear whether ε-PL consisting of 25–35 L-lysine residues is a polymer product with an original chain length synthesized by Pls, since strain NBRC14147 produces ε-PL-degrading enzymes.\(^{17}\)

During ε-PL fermentation, production and accumulation of it are detected when the pH value of the fermentation broth decreases from approximately 7.0 to a self-stabilized final value of approximately 3.2.\(^{17}\) After fermentation is complete, ε-PL can be enzymatically degraded by increasing the pH value to approximately 7.0. Such degradation is also observed using washed mycelia at pH 7.0. Nagasawa and co-workers successfully purified ε-PL-degrading enzyme Pld from S. albulus.\(^{21}\) Pld was classified as a type of Zn\(^{2+}\)-containing aminopeptidase that is tightly bound to the cell membrane and that releases N-terminal L-lysines one at a time. The mode of ε-PL degradation with Pld is exo-type. Its highest activity was observed at pH 7.0, in good agreement with the observation that the ε-PL produced was degraded at neutral pH. Furthermore, Hamano et al. have reported that the pld gene plays a partial role in self-resistance.\(^{23}\) They expected increased productivity or a change in the molecular size of the ε-PL (25–35 mer) produced on inactivation of the pld gene, but contrary to this, no alterations were observed. In addition, a high level of ε-PL-degrading activity was detected in the pld gene disruptant, while its aminopeptidase activity was significantly lower than the wild type due to inactivation of pld. ε-PL degradation was indicated to be catalyzed by endo-type peptidases, since no L-lysine monomers were released.

Recently, Yamanaka et al. identified the gene encoding the unidentified ε-PL-degrading enzyme (Pld II) with endo-type peptidase activity in S. albulus NBRC14147.\(^{25}\) Their study provides insight into the original chain length of ε-PL synthesized by Pls in vivo. Moreover, it reveals that acidic pH conditions during ε-PL fermentation are necessary for the accumulation of intracellular ATP, rather than inhibition of Pld II. The pld II gene was found to be translationally coupled with the pls gene. The primary structure of the pld II gene product showed similarity to Pld. Pld II activity was detected on the cell surface, but not in the supernatant of the culture broth, suggesting that Pld II bound to the outside of the cytoplasmic membranes. Furthermore, like Pld, the presence of Zn\(^{2+}\)-binding motif HEXXXH and the N-terminal signal peptide in the gene product was indicated by a database search.

Yamanaka et al. constructed a pld II-null mutant, CRM004, in which the pld and pld II genes were both inactivated.\(^{24}\) This mutant showed negligible ε-PL-degrading activity. It was expected that the productivity and/or the chain length of the ε-PL produced by CRM004 would be altered, because this strain lacks ε-PL-degrading activity. To confirm this, the CR1 (derivative of the wild-type strain) and CRM004 strains were cultivated in an ε-PL production medium. However, contrary to expectation, these strains showed identical ε-PL productivity and chain length. On the other hand, these findings answered the question...
regarding the original chain-length of ε-PL synthesized by Pls in vivo: Pls produces the ε-PL consisting of 25–35 L-lysine residues in vivo.

As described above, production and accumulation of ε-PL is detected when the pH value of the fermentation broth in which it is growing decreases from its initial pH value (approximately 7.0) to its self-stabilized final value (approximately 3.2), and ε-PL-degrading activity is detected mainly on the cell surface at neutral pH. The self-stabilized acidic pH condition has been believed to be an important fermentation parameter inhibiting ε-PL degradation on the cell surface, thereby resulting in its production and accumulation. However, considering that the optimum pH for L-lysine polymerization catalyzed by Pls is 8.5, the productivity and/or chain length of ε-PL in strain CRM004 should be altered in a medium with a pH higher than 3.2.

To examine the pH effect directly, strain CRM004 was incubated in citrate buffer at various pH values (3.8–5.5) (Fig. 4), because S. albulus NBRC14147 can produce ε-PL in citrate buffer containing only glucose and ammonium sulfate as carbon and nitrogen sources respectively. Similar ε-PL productivity was again observed in the CRM004 and CR1 strains, although the former showed negligible ε-PL-degrading activity (Fig. 4). The chain-length diversity of ε-PL was also unchanged. In addition, the ε-PL productivity of CRM004 fell at pH values above 4.7, indicating that S. albulus NBRC14147 does not produce ε-PL under neutral pH conditions. In general, fermentation by microorganisms is characterized by an accumulation of organic acids and an accompanying fall in pH, but organic acids are not involved in the Pls catalytic machinery. This suggests another reason why production and accumulation of ε-PL occurs under acidic pH conditions.

V. Mechanism of ε-PL Production and Accumulation

Strict culture broth pH control is essential to obtain high ε-PL productivity. Optimized fermentation was divided into two phases. In the first, cell growth was accelerated by maintaining the pH at values above 5.0, and in the second, the pH was maintained at about 4.0 to achieve the highest ε-PL productivity. Yamanaka et al. found that acidic pH conditions are not required to inhibit ε-PL-degrading enzymes, as described above. To investigate the regulatory mechanisms of ε-PL biosynthesis, S. albulus NBRC14147 was cultivated in ε-PL production medium under strict pH control (Fig. 5A), and total RNA was isolated after 9, 11, 13, 15, and 24 h (Fig. 5B). Quantitative RT-PCR (Fig. 5C) revealed that the aspartokinase (Ask) gene, which encodes the rate-limiting enzyme of L-lysine biosynthesis, was transcribed throughout the cultivation (Fig. 5C). pls
transcription was not detected in the early growth phase (~9 h), but appeared at 11 h, indicating that Pls was transcriptionally regulated to operate during the mid-log and stationary phases of growth. In this experiment, $\varepsilon$-PL production was initiated at 20 h, indicating that Pls was expressed 9 h earlier than $\varepsilon$-PL production occurred (Fig. 5A). In addition, accumulated intracellular ATP levels were observed during the stationary phase of growth.

In the Pls catalytic reaction, l-lysine molecules are polymerized via multiple enzymatic reactions: adenylation, thiolation, and peptide formation, but Yamanaka et al. demonstrated that Pls showed a typical hyperbolic saturation curve with respect to the l-lysine concentration at a fixed ATP concentration, and the $K_a$ and $V_{max}$ values for l-lysine at 30°C were calculated to be $70.9 \pm 10.7 \mu M$ and $6,607 \pm 324 \text{ pmol}^{-1} \text{s}^{-1} \text{mg}^{-1}$ respectively. A double sigmoid curve was obtained for ATP at a fixed l-lysine concentration, suggesting that Pls is allosterically regulated by ATP. Two Hill coefficients ($n_H$) were calculated from the graph: 0.72 (0.25–2 mM ATP) and 3.26 (3–5 mM ATP). Although the regulation mechanism was complicated, the affinity of Pls for ATP represented by $S_{0.5}$, which is synonymous with $K_a$ on allosteric enzymes, was low as compared with that of l-lysine (estimated to be approximately 2 mM). Moreover, considering the calculated $n_H$ values, a low concentration (0.25–2 mM) and a high concentration (3–5 mM) of ATP showed negative and positive cooperative allosteric interactions respectively, implying that Pls requires a large amount of ATP for full activity. This was strongly supported by the fermentation profiles. Although S. albidus NBRC14147 grew slowly during the stationary phase (at acidic pH), large amounts of glucose and dissolved oxygen in the medium were still consumed (Fig. 5). These results indicate that the glycolytic pathway and the electron-transport chain were still active, and that almost all the ATP generated was not used for cell growth, but instead accumulated to be still active, and that almost all the ATP generated was not used for cell growth, but instead accumulated to be used as a cofactor of Pls in $\varepsilon$-PL biosynthesis. In fact, the accumulated ATP level was observed when $\varepsilon$-PL was produced (Fig. 5A).

Taken together, these findings indicate that Pls expressed in the early growth phase (Pls was expressed 9 h earlier than $\varepsilon$-PL) is negatively regulated due to low intracellular ATP levels, thereby producing no $\varepsilon$-PL. In other words, allosteric regulation of Pls avoids excess consumption of ATP for primary metabolism in the early growth phase. Thus the concentration of intracellular ATP is a key regulatory factor for $\varepsilon$-PL biosynthesis. By contrast, considering that l-lysine affinity for Pls is relatively high, the l-lysine biosynthetic pathway should provide sufficient l-lysine for $\varepsilon$-PL biosynthesis.

VI. Industrial and Medical Applications of $\varepsilon$-PL

The safety of $\varepsilon$-PL has been confirmed by experiments using rats. In a two-generation reproduction study, the non-toxic dosage level of $\varepsilon$-PL was concluded to be 10,000 ppm, and $\varepsilon$-PL at 30,000 ppm did not cause any toxicities for reproduction, neurological function, embryonic and fetal development, or growth. The pharmacokinetics of $\varepsilon$-PL in vivo were also investigated. Absorption, distribution, metabolism, and excretion (ADME) studies using $^{14}$C-radiolabeled $\varepsilon$-PL revealed that $\varepsilon$-PL was poorly absorbed in the gastrointestinal tract, and most of the dosed radioactivity was eliminated by excretion within 168 h. Furthermore, no accumulation of $\varepsilon$-PL in any tissue or organ was observed by whole body autoradiography. Based on these results, $\varepsilon$-PL was approved by the Japanese Ministry of Health, Labor, and Welfare as a preservative for foods in the late 1980s. Afterwards, $\varepsilon$-PL was listed in the Korea Food Additives Code and has been used in Korea. Recently, the US Food and Drug Administration indicated that they had no doubts as to the manufacturer’s conclusions that $\varepsilon$-PL is to be considered generally-recognized-as-safe (GRAS) for use as an antimicrobial agent in cooked or sushi rice at levels up to 50 mg/kg of rice (USFDA 2004). In general use, because the concentration of $\varepsilon$-PL required for food preservation is low due to its strong antimicrobial activity, the taste of foods is not affected. Although $\varepsilon$-PL can be used alone for food preservation, its preservative activity of $\varepsilon$-PL is greatly improved by combining it with other food additives.

Combination of $\varepsilon$-PL and other food additives such as glycine, ethanol, organic acids including vinegar and lactic acid, and emulsifying agents are effective for multiple food preservation due to synergistic effects. For preservation of raw chicken containing a high level of microbial contaminants (4–5 Log CFU/g as aerobic bacteria), dipping treatment using 450 ppm $\varepsilon$-PL solution containing 0.9% organic acid and 1.4% sodium acetate gave a 12-d preservative effect at 4°C, 3 times longer than without treatment. The synergistic effect helps to reduce the total amount of preservatives and serves the additional positive effect of enhancing preservative activity without affecting the original food taste. $\varepsilon$-PL is used together with these additives in most cases.

Diverse amino acid polymers have been chemically synthesized to find attractive bioactive substances. Among them, $\alpha$-poly-l-lysine ($\alpha$-PL) has shown various applications in the life-science field due to its poly-cationic property. In the medical field, $\alpha$-PL is used to enhance the efficacy of some interferon inducers. It has also been reported that $\alpha$-PL enhances the drug permeability of Chinese hamster ovary (CHO) cells, and the poly-ionic complex of DNA with $\alpha$-PL is used together with these additives

As a novel application of $\varepsilon$-PL in medical use has been reported. Obesity is a serious disease that can lead to numerous health problems, including diabetes. Because it is known that pancreatic lipase plays a crucial role in lipid absorption from the intestine, natural products that inhibit pancreatic lipase activity can...
suppress dietary fat absorption. It has been reported that
highly basic proteins such as protamine, purothionin,
and histone inhibit lipase activity in vitro.\(^{31}\) Kido et al.
have reported that 10–100 mg/L of \(\varepsilon\)-PL inhibited
pancreatic lipase activity in substrate emulsions contain-
ing bile salts and phosphatidylcholine as an emulsify-
ing agent.\(^{32}\) In addition, \(\varepsilon\)-PL was found to retain
its inhibitory activity after incubation with digestive
enzymes such as trypsin, \(\alpha\)-chymotrypsin, and pepsin
due to its unusual isopeptide bonds, whereas \(\alpha\)-PL,
showing the same level of inhibitory activity, did not.
Hence, it was suggested that \(\varepsilon\)-PL would inhibit lipase in
the digestive tract, in which proteins and \(\alpha\)-PL, which
have the usual \(\alpha\)-peptide bonds, can be digested. Based
on these results, they concluded that \(\varepsilon\)-PL should be able
to suppress dietary fat absorption from the small
testine by inhibiting pancreatic lipase activity.

Lipopolysaccharide (LPS), a constituent of the outer
membrane of Gram-negative bacteria, is a potential
contaminant of physiological fluids. Because it causes
pyrogenic and shock reactions, it must be removed from
medicines that are injected. It has been reported that
endotoxins can be removed selectively using particles
prepared by cross-linking between \(\varepsilon\)-PL and chlorome-
thyloxirane.\(^{33}\) Applying this principle, a chromato-
graphy resin, \(\varepsilon\)-PL-immobilized cellulose beads (ET
clean, Chisso Corp.), was developed, and is widely used
as an endotoxin remover in some countries, including
Japan, Korea, China, India, and EU members.

A cell-culture device with \(\varepsilon\)-PL as a coating material
for the surface of devices has also been developed. It
showed low cytotoxicity and improvement in cell
adhesion.\(^{32}\) In some biomedical fields, \(\varepsilon\)-PL is also
used to prevent periodontal disease. A combination of
0.02–2.0% w/w \(\varepsilon\)-PL and 0.1–10% w/w bentonite, a
clay mineral, inhibited the production of oral bacterial
toxins.\(^{34}\) In addition, \(\varepsilon\)-PL is used as a component of the
drug solution in disposable wipes, which are used for
sanitation.\(^{35}\) Many other \(\varepsilon\)-PL applications have been
patented. In the future, further applications of \(\varepsilon\)-PL will
probably be developed.

VII. Concluding Remarks and Future Perspectives

Since the discovery of \(\varepsilon\)-PL, numerous studies of its
microbial production, antimicrobial activity, and appli-
cations have been reported. It is now industrially
produced, and is used in food and medical applications
due to its poly-cationic property and safety. Many
applications of \(\varepsilon\)-PL have been patented, and further
studies in novel applications are in progress. The water-
solubility, poly-cationic, non-toxic, edible feature and
other physicochemical properties of \(\varepsilon\)-PL are applicable
in other fields, such as agriculture and environmental
science. Recently, Pls and its gene were identified.\(^{18}\)
The identification of Pls homologs should help to create
new classes of biopolymers which ought be useful in
various applications.

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