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High Molecular Weight Poly-γ-Glutamic Acid: Synthesis, Production, and Its Application

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Poly-γ-glutamic acid (γ-PGA) is an unusual anionic polypeptide in that glutamate, mainly the D-enantiomer, is polymerized via a γ-amide linkage and has potential as a new biodegradable material in the food and cosmetics industries as well as in medicine, but a mass-production system for this useful biopolymer remains to be developed. Recently a bacterium with high γ-PGA productivity was isolated from the traditional Korean beanpaste Chungkookjang. This bacterium could be classified as a *Bacillus subtilis*, but sporulation in culture was infrequent in the absence of Mn^{2+}. It was judged to be a variety of *B. subtilis* and designated *B. subtilis* chungkookjang. *B. subtilis* chungkookjang harbors no plasmid and is the first *B. subtilis* strain reported with both naturally high γ-PGA productivity and high genetic competence.

An enzymatic system for the γ-PGA synthesis in *B. subtilis*, the PgsBCA system, was investigated. The gene-disruption experiment showed that the enzymatic system was the sole machinery of γ-PGA synthesis in *B. subtilis*. We succeeded in achieving the enzymatic synthesis of elongated γ-PGAs with the cell membrane of the *Escherichia coli* clone producing PgsBCA in the presence of ATP and D-glutamate. The enzyme preparation solubilized from the membrane with 8 mM CHAPS catalyzed ADP-forming ATP hydrolysis only in the presence of glutamate; the D-enantiomer was the best cosubstrate, followed by the L-enantiomer. Each component of the system, PgsB, PgsC, and PgsA, was translated *in vitro* and the glutamate-dependent ATPase reaction was kinetically analyzed. The γ-PGA synthetase complex, PgsBCA, was suggested to be an atypical amide ligase. The *pgsBCA* genes encoding the membrane-associated γ-PGA synthetase complex are then discussed, along with the latest information on the structure and function of the PgsBCA enzyme complex.

L-Glutamate significantly induced γ-PGA production, and highly elongated γ-PGAs were synthesized. The volumetric yield reached 35 mg ml^{-1} in the presence of 3% L-glutamate. The D-glutamate content was over 50% in every γ-PGA produced under the conditions used. During γ-PGA production, glutamate racemase activity was found in the cells, suggesting that the enzyme is involved in the D-glutamate supply.

Highly water-absorbent and biodegradable γ-PGA derivatives have potential as substitutes for hydrogels and thermoplastics synthesized from petroleum. Furthermore, γ-PGA may physiologically function as an adaptation agent in various environments: for example, neutralization of pH near the cell surface in alkalophiles, prevention of drastic dehydration under extremely high saline conditions in halophiles and nullification of immunity in infectious *B. anthracis*. γ-PGA increases Ca^{2+} solubility *in vitro* and *in vivo* and intestinal Ca^{2+} absorption. Calcium solubility *in vitro* increased with an increase in the amount of γ-PGA, due to inhibition of the formation of an insoluble complex of Ca^{2+} with phosphate by γ-PGA. Moreover the data strongly supported the notion that the average molecular weight of γ-PGA is related to the effects on Ca^{2+} solubility. Thus, γ-PGA may be important as a therapeutic tool in the treatment of osteoporosis.