Preparation of Protein-loaded Biodegradable Nanoparticles Based on Poly(γ-glutamic acid) Hydrophobic Derivatives and Their Potential Biomedical Applications

Takami Akagi[1,4], Shinsaku Nakagawa[2,4], Masanori Baba[3,4], and Mitsuru Akashi[1,4]

[1]*Department of Applied Chemistry, Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita 565-0871, Japan*
[2]*Department of Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Osaka University, Japan*
[3]*Division of Antiviral Chemotherapy, Center for Chronic Viral Diseases, Graduate School of Medical and Dental Sciences, Kagoshima University, Kagoshima, Japan*
[4]*CREST, Japan Science and Technology Agency (JST), Tokyo, Japan*

Introduction: Biodegradable polymeric nanoparticles (NP) have been widely used in biomedical applications, such as drug, gene and vaccine delivery systems. Amphiphilic block/graft copolymers consisting of hydrophilic and hydrophobic segments are self-assembling materials, and are capable of forming polymeric associates in aqueous solutions. Recently, our group developed novel biodegradable NP composed of hydrophobically modified poly(γ-glutamic acid) (γ-PGA)[1-3]. γ-PGA is a bacterially produced, water-soluble poly (amino acid)s that is the object of current interest because of its natural origin and biodegradability. In this study, we prepared protein-loaded γ-PGA NP by surface immobilization and encapsulation methods. Moreover, to evaluate the potential application for vaccine carrier, we investigated the immune responses in mice after the subcutaneous immunization with antigen-loaded NP. The interaction of NP and dendritic cells (DCs) was also investigated.

Methods: γ-PGA was hydrophobically modified by L-phenylalanine ethylester (L-PAE) in the presence of carbodiimide (WSC) (Fig. 1). The purified γ-PGA-*graft*-L-PAE was characterized by ^{1}H-NMR. NP composed of γ-PGA-*graft*-L-PAE were prepared by a precipitation and dialysis method. The various proteins were immobilized or encapsulated on/into the γ-PGA NP. C57BL/6 mice were immunized subcutaneously with ovalbumin (OVA)-encapsulated NP, and cytotoxic T lymphocyte (CTL) response was evaluated by ^{51}Cr release assay.

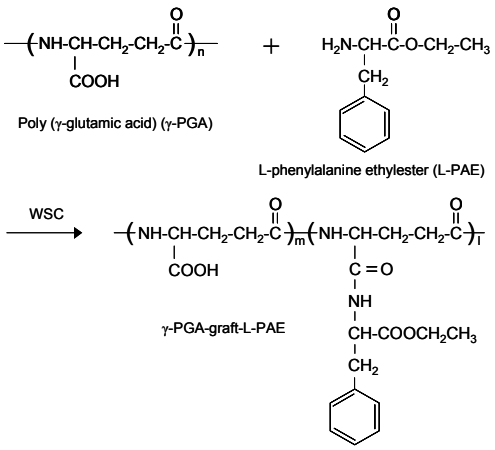

Figure 1. Synthesis of γ-PGA-*graft*-L-PAE.

Results and Discussion: γ-PGA was hydrophobically modified by chemical modification with L-PAE. In this experiment, γ-PGA-*graft*-L-PAE with 53% grafting degree was used. γ-PGA-*graft*-L-PAE could form NP due to their amphiphilic characteristics. The particle size of the γ-PGA NP was about 200 nm, and showed a highly negative zeta potential in PBS (Fig. 2). Various model proteins were loaded on/into the NP under different conditions: encapsulation, covalent immobilization and physical adsorption. The encapsulation method showed the most promising results for protein loading. Protein loading onto the NP was also influenced by electrostatic interaction. The NP are internalized efficiently by DCs (Fig. 3) and that uptake in concentration- and time-dependent. Subcutaneous immunization with OVA-encapsulated NP could induce effective CTL responses. This result suggests that the γ-PGA NP provides an efficient vaccine delivery system for the induction of cellular immunity and the development of retroviral vaccine.

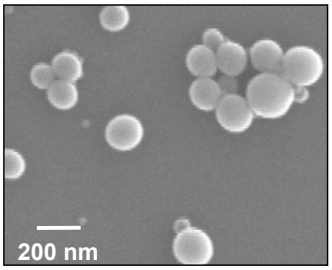

Figure 2. SEM image of γ-PGA NP.

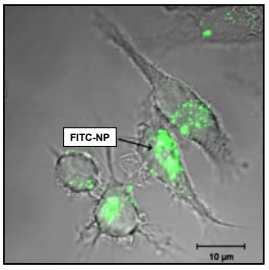

Figure 3. Uptake of γ-PGA NP by DCs.

Conclusions: It is expected that biodegradable γ-PGA NP can encapsulate and immobilize proteins, peptides, plasmid DNA and drugs. These multifunctional NP have great potential as carriers for biomolecules. The protein-loaded γ-PGA NP may be considered promising biodegradable and biocompatible protein carriers for modulated biodistribution, and site and/or cell-specific vaccine delivery systems.

References: [1] Matsusaki M. et al. Chem Lett. 2004;33:398-399. [2] Kaneko T. et al. Chem Mater. 2005;17:2484-2486. [3] Akagi T. et al. Macromol Biosci. 2005;5:598-602.