Intracellular degradation and distribution of size-controlled antigen-encapsulated biodegradable nanoparticles

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Statement of Purpose: The drug delivery system which employs polymeric nanoparticles (NPs) has been widely studied to develop safe and effective vaccines. The polymeric NPs have the advantage of being able to regulate their physiochemical properties, such as particle size[1)], shape, surface charge and polymer composition. Also these NPs can deliver antigens selectively to antigen-presenting cells (APCs) and induce effective immune responses. In particular, it has revealed that the size of polymeric NPs is the key factor to control antigen specific immune responses. Although there is still unclear about relationship between intracellular behavior and the size of polymeric NPs. Elucidating intracellular behavior of polymeric NPs is one of the most important issues in the development of effective vaccines. In this study, we prepared size-controlled protein-encapsulated NPs composed of hydrophobically-modified poly(γ-glutamic acid) (γ-PGA). To evaluate their intracellular behavior, the size effect on cellular uptake and intracellular degradation of proteins encapsulated in NPs were investigated. The size tunable NPs were found to be promising candidates for polymeric NPs vaccines.

Methods: γ-PGA (*M*w=3.8×10^5, D:L ratio=6:4) was hydrophobically modified by L-phenylalanine ethylester (Phe). In this study, γ-PGA-Phe with 50 Phe per 100 glutamic acid units of γ-PGA was used (**Fig. 1**). To prepare size-controlled protein-encapsulated NPs, γ-PGA-Phe copolymers were dissolved in DMSO at a concentration of 10 mg/ml, followed by the addition of 875 μg/ml OVA solutions to the same volume as DMSO. The size of the protein-encapsulated NPs was regulated by dissolving OVA in 0 or 0.1 M NaCl solution. The resulting solution was then dialyzed against distilled water to remove the DMSO. To evaluate the size effect on cellular uptake, RAW264 cells were incubated with various endocytosis inhibitors, then measured cellular uptake of different-sized protein-encapsulated NPs. Also the intracellular degradation and distribution of proteins encapsulated in NPs was evaluated by fluorescence microscope observation.

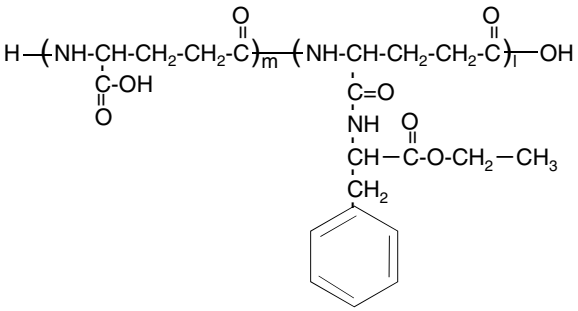

Fig. 1. Chemical structure of γ-PGA-Phe.

Results: The size of protein-encapsulated γ-PGA-Phe NPs could be easily controlled by NaCl concentration added to γ-PGA-Phe. The mean diameters of the protein-encapsulated γ-PGA-Phe NPs were 40 nm and 200 nm. By inhibiting various endocytosis, it revealed that both size of protein-encapsulated NPs were taken up by mainly clathrin- and caveolae-mediated endocytosis (**Fig. 2**). Evaluation of intracellular degradation of proteins revealed that proteins encapsulated in NPs were less degraded than those fed alone (**Fig. 3**). Moreover, the degradation of protein in NPs was affected by the size of NPs. Also encapsulated proteins were escaped from endosomes after ingested into cells. These results suggest that size-controlled protein-encapsulated NPs can efficiently deliver proteins to cells, and let them escape from endosomes.

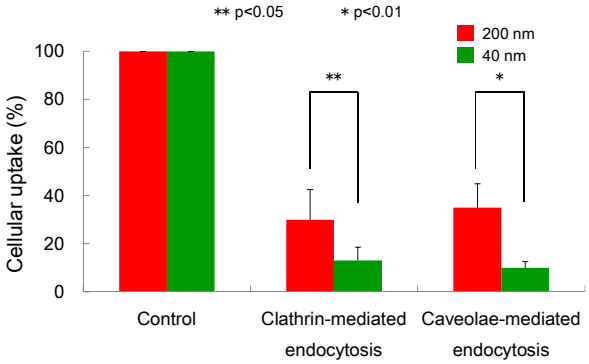

Fig. 2. Cellular uptake of protein-encapsulated NPs.

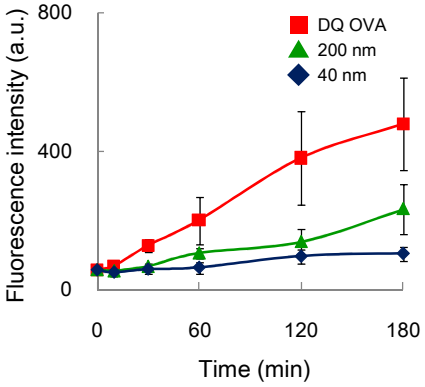

Fig. 3. Intracellular degradation of proteins encapsulated in NPs.

Conclusions: Size-regulated protein-encapsulated γ-PGA-Phe NPs have significant potential as an antigen carrier. The protein encapsulated in size-controlled NPs can efficiently escape from endosomes. The result would provide guidelines for effective adjuvant designing and the development of an effective vaccine.

References:

1) T. Akagi et al., Polymer 2007; 48: 6729-6747