Intracellular Behavior of Biodegradable Dextran-graft-oligo(lactide) Nanogels Collapsing under Reductive Condition in Cytosol for Efficient Cellular Drug Delivery

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Introduction: Nanogels are nanometer sized hydrogel nanoparticles (diameter < 1 μm) with three-dimensional networks of cross-linked polymer chains. It is well known that some hydrophobically modified water-soluble polymers can form physically crosslinked nanogels in dilute aqueous solution. Such nanogels have attracted growing interests over the decade because of their potential for applications in biomedical fields such as drug delivery systems (DDS) and bioimaging.[1, 2] Akiyoshi and coworkers reported hydrophobized polysaccharides that can form physically crosslinked nanogel in aqueous solution[1] and entrap both of hydrophobic and hydrophilic molecules such as proteins. Physical entrapment and the release behavior of proteins into and from nanogels have been investigated for application as DDS carriers.[3] Biodegradability is a crucial factor for DDS carriers. Biodegradation offers several advantages, such as facilitation of the sustained release of encapsulated molecules and excretion of the vehicles after the release. We also developed biodegradable Dex-glaft-oligo(L-lactide) (Dex-*g*-OLLA) nanogels. In addition, we succeeded the preparation of protein-loaded Dex-*g*-OLLA nanogels using lysozyme as a model protein and found that the lysozyme-loaded nanogels showed sustained release of lysozyme for 1 week without denaturation in PBS at 37 ºC. [4,5] In this study, to achieve more efficient cellular drug delivery, we prepared nanogels composed of dextrans attaching oligolactide (OLA) chains via disulfide bond to be collapsed under reductive condition in cytosol, and investigated their intracellular behavior. (Fig. 1)

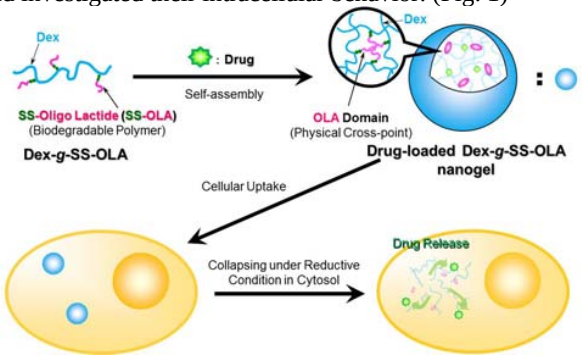

Figure 1. Schematic illustration of concept for drug delivery of Dex-g-SS-OLA nanogel.

Methods: Hydroxy-terminated OLA (OLA-OH) was prepared according to literature. The disulfide linker was introduced on the obtained OLA-OH using cystamine derivative to give OLA-SS-NH_2. Dex-*g*-SS-OLA was synthesized by coupling reaction of dextran (MW: 43,000) with OLA-SS- NH_2 using carbonyldiimidazole (CDI). Dex-*g*-SS-OLA nanogels could be prepared by a solvent exchange method using dialysis membrane. Critical aggregation concentration (CAC) values, particle sizes of the obtained Dex-g-SS-OLA nanogels were investigated by fluorescence probe method, dynamic light scattering (DLS) and atomic force microscope (AFM). Cell viability and cellular uptake of Dex-g-SS-OLA nanogels were carried out by using L929 and HepG2 cells, respectively.

Results: Dex-*g*-SS-OLA was synthesized by coupling reaction of dextran with OLA-SS-NH_2 using carbonyldiimidazole. The degree of polymerization of L-LA was 20, average number of OLA chains per dextran molecule was 8.0, and sugar content of Dex-g-SS-OLA is 64wt%. Dex-*g*-SS-OLA nanogels could be prepared by a solvent exchange method using dialysis membrane. CAC of Dex-*g*-SS-OLA in aqueous solution was determined by using pyrene as a fluorescence probe to be a relatively low value. Hydrodynamic diameter of the nanogel was estimated by DLS and AFM to be ca. 150 nm. In the presence of DTT, hydrodynamic diameter decreased from 150 nm to around 10 nm. On the other hand, in the absence of DTT, the diameter did not change. These results suggest that collapsing of Dex-g-SS-OLA nanogels was confirmed under reductive condition. Dex-*g*-SS-OLA nanogels have no cytotoxicity. To deliver the nanogel into cytosol, Dex-*g*-SS-OLA nanogels were modified with galactose residues and oligoamines for receptor-mediated endocytosis and endosomal escape, respectively. The modified nanogels were taken into HepG2 cells by introduction of specific ligands and released from an endosome by protone-sponge effect.

Conclusions: In conclusions, Dex-*g*-SS-OLA could successfully be synthesized. CAC of Dex-*g*-SS-OLA in aqueous solution was determined by using pyrene as a fluorescence probe to be a relatively low value. Hydrodynamic diameter of the nanogel was estimated to be about 150 nm by dynamic light scattering and atomic force microscope measurements. Moreover, collapsing of Dex-*g*-SS-OLA nanogels was confirmed in the presence of reductive agent by monitoring hydrodynamic diameter changes. Dex-*g*-SS-OLA nanogels have no cytotoxicity and were taken into HepG2 cells by introduction of specific ligands. The nanogel released from an endosome by protone-sponge effect. The biodegradable nanogel collapsing under reductive condition in cytosol is expected to release the drug after uptaking into cells, and should have great potential as a drug delivery carrier.

References: (1) Morimoto, N. et al., Nanotechnol Carbohydr Chem 2006; 67. (2) Otsuka, H. et al., Adv Drug Delivery Rev 2003; 55: 403. (3) Ayame, H. et al., Bioconj Chem 2008; 19: 882. (4) Nagahama, K. et al., Biomacromolecules 2007; 8: 2135. (5) Nagahama, K. et al., Macromol. Biosci. 2008; 8: 1044.