Nanoparticles formed from the interaction between chitosan and proteins for encapsulation and delivery of hydrophobic molecules

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論 文 名　：Nanoparticles formed from the interaction between chitosan and proteins for encapsulation and delivery of hydrophobic molecules
（キトサンとタンパク質の複合化により形成されるナノ粒子への疎水性分子の封入と送達に関する研究）

区　　分　：甲

論 文 内 容 の 要 旨

In the past two decades, nanotechnology has generated great attention of researchers in various fields. In the fields of food, pharmacy and medicine, nanoparticles (NPs) are considered a promising material for a creation of new therapy for many diseases, especially cancer and degenerative diseases. The application of NPs in the biological systems requires a careful selection of constituting materials for fabrication of NPs in terms of cost, availability, and safety. Due to their biodegradability, biocompatibility, and low immunogenicity, proteins and polysaccharides are attractive materials for development of NPs for effective cancer treatment. In this thesis, we investigated the formation of NPs between proteins and chitosan (CH) and evaluated their feasibility for encapsulation and delivery of hydrophobic therapeutic molecules.

In Chapter 1, we described the general introduction on this topic, which includes the advantages of NPs and the formation of NPs based on the assembly of proteins and polysaccharides.

In Chapter 2, we described the formation of electrostatic-based nanocomplexes (NCs) between caseinate (CS) and chitosan (CH) (CCNCs) and evaluated the ability of these NCs for encapsulation of curcumin. The particle size, polydispersity index (PDI), turbidity, number of particles, and zeta potential of these NCs were found to be dependent on the total biopolymer concentration and the concentration of CS/CH in these NCs. Higher CS concentrations resulted in higher turbidity and lower number of particles and zeta potential, at least in the range studied. The obtained particle size was less than 400 nm with narrow size distribution and positive charge. In addition, the interaction between CH and CS was confirmed by FT-IR spectroscopy. The ability of these NCs to encapsulate curcumin was demonstrated and verified using UV-Vis and fluorescence spectrophotometer. However, the encapsulation efficiency (EE) of curcumin was moderate, at 56%. This might be due to the presence of free CS/CH and soluble complexes that competes with the NPs. In view of practical application, the water re-dispersibility of these curcumin-loaded CCNCs was tested using a lyophilized form with 5% trehalose as a cryoprotectant. We showed that CCNCs can enhance thermal and storage stability as well as re-dispersibility of curcumin in water. Of interest, CH solubility in water

was promoted after freeze-drying, which suggests favorable interactions between CH and trehalose.

In Chapter 3, we focused on enhancing the stability of CS/CH NPs (CCNPs) described in the previous chapter by a non-toxic cross-linker, genipin and evaluated the feasibility of genipin-crosslinked CCNPs (G-CCNPs) for curcumin delivery into the cells. DLS and TEM analysis revealed that G-CCNPs were more stable under acidic and neutral pH at physiological conditions as compared with NPs without cross-linking. The biocompatibility of G-CCNPs was evaluated on normal fibroblast cells (L929), and WST assay revealed that these NPs were non-toxic. Curcumin was delivered by G-CCNPs to both cancer (HeLa and A549) and normal cells (L929). Cytotoxicity *in vitro* of curcumin on cancer cells was improved by G-CCNPs. We found that this enhanced bioactivity of curcumin was not necessarily correlated with enhanced cellular internalization of NPs into the cells, as most G-CCNPs were located on the cell membrane. Rather, the stability of curcumin in the physiological condition was more important.

To eliminate the use of a cross-linker agent and overcome the limitation of CH, in Chapter 4, we fabricated NPs formed from the complexation between reduced bovine serum albumin (rBSA) and glycol chitosan (GC) (rBG NPs) and evaluated their feasibility as a nano-carrier for paclitaxel (PTX). We found that the degree of complexation between rBSA and GC was highly dependent on rBSA/GC mass ratio and pH. The optimum complexation was found to be at higher rBSA/GC mass ratio or at higher pH (6.5). Our results indicated that rBG NPs formation was governed by the initial rBSA aggregation in the solution, followed by the electrostatic complexation with GC to form NPs with core-shell structure. Moreover, rBG NPs had excellent stability without the help of external cross-linker agent. Hydrophobic and disulfide crosslinking are presumably responsible for stabilizing these NPs. We showed that PTX can be efficiently encapsulated into these NPs. Unlike G-CCNPs, these NPs can be effectively internalized into the cells, which suggests that cross-linking reaction based on primary amines can affect colloidal stability and cellular internalization of NPs. Cytotoxicity *in vitro* revealed that PTX-loaded in rBG NPs had comparable anti-cancer activity compared with Taxol-like formulation at a longer time of incubation (48 h), suggesting that PTX was released slowly due to strong binding with these NPs, presumably via hydrophobic interactions. The findings from these studies suggest that nanostructures fabricated from chitosan-proteins interaction could be used as a novel soft biomaterial for bioactive delivery system.

In Chapter 5, we summarized the findings of this research and briefly discussed further research directions related to this research.

〔作成要領〕

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