Water-Soluble Low Molecular Weight Chitosan for Plasmid DNA Delivery Vehicles

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Introduction

For gene therapy, viral delivery system is currently the most effective way. However, it introduces serious concerns about endogenous virus recombinations, oncogenic effects, and immunological reactions. Such concerns have limited the use of viral vectors for human gene therapy(1). These limitations prompted the development of non-viral delivery systems(2), its have several advantages to viral systems. They can accommodate large size DNA, be modified with appropriate ligands for specific cell targeting, and be administered repeatedly. However, non viral system has its limitations such as toxicity and low transfection efficiency. To reduce the cytotoxicity and increase transfection efficiency, several kinds of derivatives of poly-L-lysine have been synthesized. These modifications include conjugation of asialoglycoprotein, transferrin, antibody, lactose, and mannose(3, 4, 5).

Chitosan has also been attractive gene carrier, because of its high positive charges and low toxicity to cell(6). In this study, water-soluble depolymerized low molecular weight chitosan(LMWC) was characterized and evaluated as a gene carrier.

Materials and methods

Depolymerized LMWC derivatives

The deamination of chitosan (Fluka: Deacetylation degree, 85 %) was performed according to the previous method(7). Crude chitosan oligomer mixture, depolymerized by nitrous acid, was filterated to remove non-soluble high molecular chitosan. The filtrate was isolated by UF (Ultra filtration) method (Amicon Co., cut-off size: 500, 1,000, 3,000 and 10,000). In order to gain LMWC, each isolated solution was precipitated in methanol. These LMWC precipitants were washed with acetone and ethyl ether and dried *in vacuo*

at 40℃ for 48 hours.

The moleculular weight

The molecular weight of depolymerized chitosan derivatives was conducted by GPC with Universial method: Asahi Pack GFA-30F column, 50℃, 0.5 ml/min 0.5 % acetate buffer (pH 4.0), RI detector (SP6040 Differential Refractometer Electronics Unit), pullulan standard.

Preparation of pGL3 Plasmid

pGL3 plasmid was introduced into E.coli strain DH5α, and purified by Qiagen Plasmids Maxi kit. Purity of plasmid DNA was certified by OD260/OD280 ratio, and by distinctive bands of DNA fragments at corresponding base pairs in gel electrophoresis after restriction enzyme treatment of the DNA. The concentration of plasmid DNA was determined using 1(OD260)=50㎍ of DNA. Plasmid DNA was stored at -20℃ until use.

Preparation of Plasmid/LMWC Complex and Gel Retardation Assay

Plasmid/LMWC complexes were prepared by self-assembly. Various amounts of LMWC was slowly dropped into 1㎍ of the plasmid DNA and left for 30 minutes at room temperature for complex formation. The complexes were electrophoresed on 1%(w/v) agarose gel for 60 minutes at 80V. The gel was stained with ethidium bromide(0.5㎍/㎖) for 30minutes and illuminated on an UV illuminator to show the located of the DNA.

Results and discussion

Chitosan was partially degraded by using nitrous acid. The deacetylation degree of chitosan decreased with its degree of hydrolysis. The deacetylation degree and molecular weight of fractionated degraded products were shown Table 1. The degraded products were fractionated by UF membrane (cut-off size: 500, 1,000, 3,000 and 10,000) had lower deacetylation degree and narrower polydispersities than those by precipitation method. LMWC is highly water soluble, and can form complex with plasmid in physiological buffer.

Table 1. The deacetylation degree and molecular weights of each fractions of partially hydrolyzed chitosan separated by UF membrane.

Used Membrane Filters (Mw)	Deacetylation degree (%)	Mn	Mw/Mn
YC05 (500)	18	1,200	1.18
YM1 (1000)	25	1,800	1.20
YM3 (3000)	32	3,100	1.25
YM10 (10000)	47	5,300	1.52

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