수용성 키토산 유도체의 제조와 유전자 전달체로서의 특성

장미경, 김동곤, 김태형[1], 나재운[*]
순천대학교 고분자공학과, [1]조선대학교 내성세포연구센터
(jwnah@sunchon.ac.kr[*])

Characterization as Gene Carrier and Preparation of Water-Soluble Chitosan Derivatives

Mi-Kyeong Jang, Dong-Gon Kim, Tae-Hyeong Kim[1], and Jae-Woon Nah[*]
Department of Polymer Science and Engineering, Sunchon National University
[1]Research Center for Resistant Cells, Chosun University College of Medicine
(jwnah@sunchon.ac.kr[*])

Introduction

Gene therapy provides significantly important opportunities to treat various kinds of life-threatening and gene related-disease by producing biologically active agents or stopping abnormal functions of the cells such as genetic failure or uncontrollable proliferation of cells [1]. Actual application of genes to human therapy is limited by several problems, including their instability in body fluids, non-specificity to the desired cells, degradation by nucleases, and low transfection efficiency. Gene delivery systems have been investigated in attempt to enhance the gene expression and reduce the cytotoxicity [2]. Cationic polymers including poly(L-lysine) (PLL) and polyethylenimine (PEI) were able to condense plasmid DNA and protect it from enzymatic degradation, which results in the enhancement of the transfection efficiency. However, there still remain drawbacks such as biocompatibility in body. To overcome the biocompatibility problem, non-toxic biodegradable polymeric gene carrier has been developed as a promising gene delivery materials [3,4].

Chitosan, biocompatible natural polymer, was first described as a delivery system for plasmids by Mumper et al. [5]. So for, several gene delivery trials have been made with chitosan. Oral gene delivery with chitosan-DNA nanoparticles was also tried.

In this chapter, water-soluble chitosan (WSC) prepared by novel method [20] and its derivatives were characterized and evaluated as a gene carrier. WSC is highly water soluble and can form complex with plasmids in physiological buffer. Functional group introduced in amine group of WSC can be evaluated the ability of complex form with DNA. To investigate the potential as a gene carrier, we evaluated gel retardation assay, transfection efficiency in HCT 116 cells. Also, the cytotoxicity of LMWSC also was determined by MTT assay.

Experimental

WSCs with molecular weight of 10K and 18K prepared by previous method were supplied by KITTOLIFE Co., Seoul, Korea. pSV-β-galactosidase plasmid (Promega, Madison, WI) was introduced into *Escherichia coli* strain DH5 (Gibco-BRI, Gaithersburg, MD), and purified by Qiagen Plasmids Maxi Kits (Qiagen, Valencia, CA). Methoxy poly(ethylene glycol) p-nitrophenyl carbonate (MPEG-pNP)(Mw : 5000) was purchased from Sigma Co. and cholesteryl chloroformate was purchased from Aldrich Co. Dialysis tubing (MWCO 12 000) was obtained from Spectrum. Dimethylformamide (DMF) of reagent grade was used without further purification and double distilled water was used in all of the experiments.

DNA/WSCs and its derivatives complexes were prepared by self-assembly. Various amounts of WSCs and its derivatives were slowly dropped into 1 ug of the plasmid DNA and left for 30 min at room temperature for complex formation. For gel retardation assay, DNA/LMWSCs complexes were electrophoreded on 1% (w/v) agarose gel for 60 min at 80 V. The gel was stained with ethidium bromide (0.5 ìg/ml) for 30 min and illuminated on an UV illminator to show the location of the DNA. For the transfection studies, 293T cells, a human kidney cell line, were seeded at a density of 2 × 106 cells/dish in 100-mm culture dishes, and incubated for 24 hrs before the addition of the plasmid/polymer complex. Transfection assay was performed by method described in the previous study[6].

Results and discussion

WSCs having various molecular weight and its derivatives were evaluated as a gene carrier due to its strong positive charge. In the previous reports, it was already revealed that chitosan can form complex with plasmid DNA. In this study, we prepared WSC derivatives and investigated the ability to complex with DNA, transfection efficiency, and cytotoxicity etc. First, WSCs was more superior than its derivatives in the form ability of complex with DNA. Because WSC derivatives were decreased amine group at glucosamine unit as binding with function group. Therefore, DNA/WSC 18K complex was formed above at 1:2 weight ratio. However, WSC derivatives modified with MPEG was completely retarded above at 1:16 weight ratio [Figure 1].

From transfection results, WSC 10K was showed higher transfection efficiency than WSC 18K, but their derivative was a contrast to this result. In case of WSC, WSC 10K showed higher transfection

efficiency due to the low molecular weight. However, WSC derivatives modified with MPEG had low transfection efficiency due to the decrease of positive charge. Finally, cytotoxicity of WSC and its derivatives was negligible (Figure 2).

(a) LMWSC 18K **(b) LMWSC derivatives**

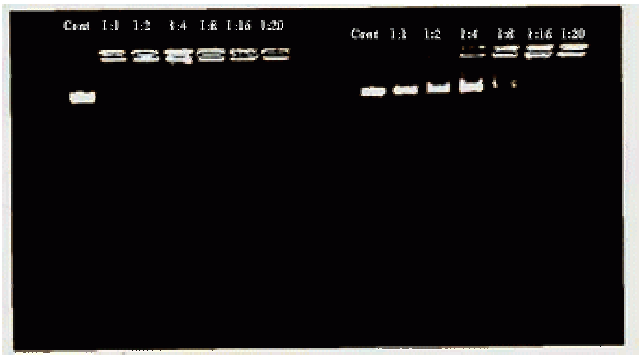

Figure 1. Gel retardation of LMWSC and LMWSC derivatives

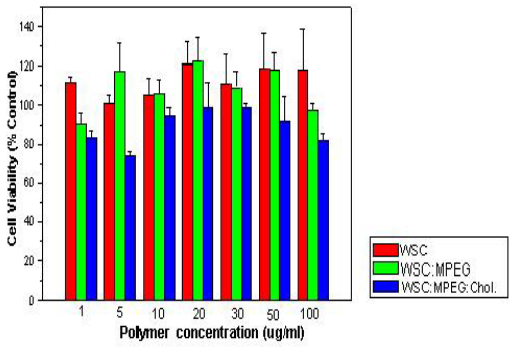

Figure 2. Cytotoxicity of WSC and its derivatives in HCT 116 cell

Conclusion

WSC was formed compex with DNA above 1:8 weight ratio, but WSC derivatives was formed higher weight ratio than itself, above 1:16. It was considered that the positice charge of WSC derivatives were declined due to substitute with MPEG or cholesterol in amine group of glucosamine unit. In the transfection test, WSC 10K was showed higher transfection efficiency than WSC 18K, but its derivatives was a contrast to this result. In cytotoxicity test, all WSC and its derivatives complexes showed negligible cytotoxicity for 293T cells. Therefore, WSCs having free amine group will be useful in the development of safe gene carriers.

References

1. Huang L., Hung M.C and Wagner E., *Nonviral Vectors for Gene Therapy*, Academic press, New York, 4(1999).
2. Liu W.G. and Yao K.D., "Chitosan and its derivatives-a promosing non-viral vector for gene transfection", *J. Control. Release*, 83, 1(2002).
3. Behr J.P., "Synthetic gene-transfer vectors", *Acc. Chem. Res*., 26, 274(1993).
4. Wagner E. and Cotton M., "Non-viral approaches to gene therapy", *Curr. Opin. Biotechnol*. 4, 705(1993).
5. Mumper R.J., Wang J.J., Claspell J.M. and Rolland A.P., "Novel polymeric condensing carriers for gene delivery", *Proc Int. Symp. Control. Rel. Bioact. Mater.*, 22, 178(1995).
6. Lee M., Nah J.W., Kweon Y., Koh J.J., Ko K.S. and Kim S.W., "Water-Soluble and low molecular weight chitosan-based plasmid DNA-delivery", *Pharmaceutical Research*, 18, 427(2001).