Ordered Fragmentation of pDNA induced by PEG-PLL block copolymer -Condensation degree and Biological Activity by the Cell-Free System-

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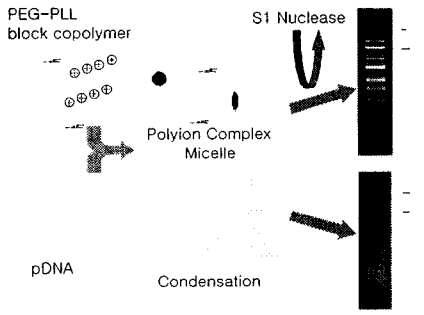
Introduction

DNA is delivered in a condensed state by complexation with appropriate cationic carriers, such as lipids and polymers, in gene delivery systems for the gene therapy. So many efforts have been performed to develop promising carrier systems, whereas structures and properties of the packaged DNA are little understood. For the sake of making the gene therapy a success, it is necessary to understand the DNA condensation properly. DNA is inherently a rod molecule due to formation of double-helical structure with a persistent length of approximately 50 nm, How does this rigid DNA fold and condense? In other word, does the DNA condense with keeping the double-helix all along the length, since denaturation of it spontaneously leads exposure of the bases, which must be biologically critical happening. Alternatively, how does the degree of condensation influence to biological activities? Understanding of the condensation should provide most proper designs of carrier system as well as elucidation of condensation mechanism within the organisms.

DNA condensation was investigated through supramolecular assembly between plasmid DNA (pDNA) and the synthetic block copolymer poly(ethylene glycol)-*b*-poly(L-lysine), PEG-PLL, comprises a cationic DNA binding segment and non-ionic hydrophilic segment. The polyion complex (PIC) micelle systems with block copolymers have been shown their feasibility for the in vivo usage[1], while in addition, they can be strong tools for studying DNA condensation systematically due to their significant characteristics derived from a distinct core-shell architecture. Since the outer PEG shell screens out intercomplex association, single condensed DNA can be obtained without causing aggregation. Using the PIC micelle system condensation process of pDNA was studied focusing on whether the double-helix form is maintained or broken under in the condensates. As a tool to detect whether the double-helix is broken, a S1 nuclease, which is known to cleave a single-strand DNA, was used. The study herein demonstrated allows proposing that DNA inherently retains a mechanism and order for folding the rigid rod molecule as well as to propose a concept that controlling the degree of condensation would be a critical issue in designing a comprehensive gene delivery system.

Results and discussion

The sensitivity of plasmid DNA to S1 nuclease was dramatically modulated through supramolecular assembly with the block copolymer. In this evaluation, if a denaturation happens at random sites every place DNA folds, the nuclease would cleave DNA into pieces, whereas if DNA folds without causing denaturation, no fragments would be generated. The incorporated pDNA with a charge ratio (nucleotides/phosphates=N/P ratio) higher than 4, which was confirmed as sphere condensates from the AFM observation, was digested in non-specific manner. In contrast, the stoichiometrically condensed pDNA, which were rod and toroid condensates, were cleaved into 7 ordered fragments each being 10/12, 9/12, 8/12, 6/12, 4/12, 3/12, and 2/12 of the original pDNA length[2]. It should be noted that the regular fragmentation universally occurred at least in any examined pDNAs ranging from 2200bp to 12000bp. The S1 digestion results indicate that denaturation of the double helix happens at non-specific sites in tightly condensed DNA, while it arises at ordered sites in moderately condensed DNA. These results suggest there must be any folding mechanisms and order depending on the degree of condensation. The differences in condensation behavior are suspected



Scheme Sensitivities of condensed pDNA by the PEG-PLL block copolymer to S1 nuclease. The nuclease cleaves the moderately condensed pDNA into 7 ordered fragments, on the other hand, it digests the tightly condensed pDNA into non-specific manner.

to affect their biological activity. Gene expression activity was then evaluated using the cell-free transcription-translation system, which reflects the intracellular conditions. The cell-free system allows evaluating correlations between degree of condensation and gene expression because the influences of size difference and surface potential, which affect critically in the conventional transfection, can be excluded. The degree of condensation showed much influence to the expression that no gene expression was observed from the tightly condensed pDNA, moreover, accelerated expression was observed for the complexes below the stoichiometric ratio.

These results suggest that control of the condensation is one of the most crucial issues in designing the brilliant gene delivery system, alternatively, giving hints for DNA condensation in nature.

Conclusion

Using the synthetic block copolymer, condensation of the pDNA was systematically studied focusing on the double-helix formation. The ordered fragmentation was commonly observed for the condensed pDNA in stoichiometric ratio regardless of species and length of pDNA by the treatment of the S1 nuclease. In contrast, the nuclease digested highly condensed pDNA (N/P ratio > 4) in non-specific manner. The results demonstrated here suggest the rigid double-helical DNA may inherently have the specific mechanism and order for folding.

References

[1] Osada K.; Kataoka K *Adv. Polym. Sci.* **2006** *202*, 113-153

[2] Osada K.; Yamasaki Y.; Katayose S.; Kataoka K. *Angew. Chem. Int. Ed.* **2005**, *44*, 3544