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論文 / 著書情報
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題目(和文)	合成高分子のコイル・グロビュール転移に基づくsiRNA 周囲の空間支配と薬理機構制御への展開
Title(English)	Develoment of a Methodology for an Artificial Control of siRNA Bioactivity Based on the Coil-Globule Transition Behavior of the Conjugated Polymeric Molecule
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種別(和文)	論文要旨
Type(English)	Summary

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（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

専攻： Department of	化学環境学	専攻	申請学位（専攻分野）： Academic Degree Requested	博士 Doctor of	（Engineering ）
学生氏名： Student's Name	Noor Faizah Binti Che Harun		指導教員（主）： Academic Advisor(main)	伸宏西山	
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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Since the discovery of RNA interference (RNAi) in mammalian cells, tremendous efforts have been devoted to the design of small interfering RNA (siRNA)-based medicine because of its strong gene silencing ability in a sequence-specific manner. siRNA-based medicine has been employed as a promising therapy for cure of several intractable diseases including cancer. Recently, it has been reported that siRNA modification improves inherent siRNA bioactivity and endows siRNA with entirely new functionalities; for example, conjugation with lipids and peptides enables facilitated cellular uptake and improved target specificity, respectively. In general, gene silencing is initiated with the recognition of siRNA by gene silencing related protein of Ago2 in the cytoplasm. Ago2 is the main catalytic component inside RNA-induced silencing complex (RISC), i.e., Ago2 will employ asRNA in siRNA and packages the asRNA to form activated RISC. Then, asRNA will bind to its complementary sequence in mRNA and cleave the mRNA. In this regard, siRNA conjugation with polymeric molecule having a steric hindrance effect can suppress siRNA recruitment into gene silencing related pathway. In the present study, I created a new siRNA-polymer conjugate for the development of a new methodology for an artificial control of siRNA bioactivity in the cell, based on coil-globule transition behavior of the conjugated polymer. The coil-globule transition of the conjugated polymer would control the induction of siRNA bioactivity, due to the considerable change of size. In order to realize the methodology, I established original synthetic roots for desired siRNA-polymer conjugates, and investigated the steric hindrance effect of the conjugated polymers on vicinal siRNA molecule, from physicochemical and biological standpoints.

In design, I linearly conjugated a thermoresponsive polymer, poly(*N*-isopropylacrylamide) (PNIPAAm) with an siRNA molecule. PNIPAAm with controlled structure was prepared through

reversible addition-fragmentation chain transfer (RAFT) polymerization, and the subsequent modification of the terminus to obtain dibenzocyclooctyl-terminated PNIPAAm (PNIPAAm-DBCO). The obtained polymer had an average molecular weight of 39,000 g/mol with molecular weight distribution of 1.24, as characterized using size exclusion chromatography (SEC) and ^{1}H NMR. Subsequently, PNIPAAm-DBCO was linearly conjugated with an siRNA molecule through copper-free click chemistry reaction in 10 mM HEPES buffer (pH 73), and the subsequent purification through ion-exchange HPLC to produce PNIPAAm-siRNA. Poly(ethylene glycol) (PEG)-siRNA was also prepared in a similar manner as a non-thermoresponsive control.

The lower critical solution temperature (LCST)-related behavior of PNIPAAm-siRNA was investigated its hydrodynamic diameter using fluorescence correlation spectroscopy (FCS) at room temperature and 37 °C for concentration of siRNA at 100 nM. As the results, hydrodynamic diameter of PNIPAAm-siRNA decreased from 7.61 nm at room temperature to 6.50 nm at 37 °C due to coil-globule transition behavior of the conjugated PNIPAAm segment. Meanwhile, unconjugated-siRNA and PEG-siRNA maintained their hydrodynamic diameter regardless of a change in temperature. In addition, thermoresponsive behavior of PNIPAAm-siRNA was investigated by evaluation of temperature-dependent light scattering intensity analysis for concentration of siRNA at 5 μM. Upon heating, the PNIPAAm-siRNA solution started to aggregate at 35 °C, which is close to the original LCST of PNIPAAm (~33 °C), confirming that PNIPAAm segment undergoes LCST-related behavior even in the presence of the vicinal siRNA molecule.

Next, I investigated the temperature-dependent gene silencing activity of PNIPAAm-siRNA for cultured human cervical cancer cells stably expressing luciferase (HeLa-Luc) at 30 and 37 °C. PNIPAAm-siRNA treatment using Lipofectamine RNAiMAX at 37 °C achieved ~80% silencing of luciferase expression, while both PNIPAAm-siRNA treatment at 30 °C and PEG-siRNA treatment at 37 °C resulted in ~20% silencing. It should be noted that no significant cytotoxicity was observed for the treatment from any samples at both temperatures, as well as no significant luciferase suppression by siScr, confirming a sequence-specific gene silencing. Hence, the enhanced gene silencing of PNIPAAm-siRNA system at 37 °C would be attributed to the events inside the cell.

To further elucidate the underlying mechanism in the temperature-dependent gene silencing for PNIPAAm-siRNA system, I investigated Ago2 interaction with siRNA and siRNA polymer conjugates by counting the number of Ago2-associated asRNA in the cells through immunoprecipitation method. The number of Ago2-associated asRNA per cell for the treatment at 37 °C was 2.84 higher than treatment at 30 °C, and this multiplied increase is higher compared to unconjugated siRNA system (1.84). This result suggests that the thermoresponsive behavior of the PNIPAAm segment, as well as the thermal effect on the cell activity, affected gene silencing efficacy. In conclusion, the conjugation of siRNA with the polymer having coil-globule transition behavior successfully controls the efficacy of the siRNA recruitment into gene silencing pathway, leading to the success in the manipulative gene silencing activity. This is the first study to represent a successful scientific methodology for an artificial control of siRNA bioactivity in the cells using excluded volume effect of the conjugated polymer. The achievement of the newly developed methodology would provide a new trend for molecular design of siRNA-based medicine and associated therapeutics.

備考 ： 論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 1 部提出してください。
Note : Thesis Summary should be submitted in either a copy of 2000 Japanese Characters and 300 Words (English) or 1copy of 800 Words (English).