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| Title | 核酸編集に向けた超高速光架橋を用いたDNAおよびRNA操作法の開発 |
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**論文の内容の要旨**

## Introduction

The Human Genome Project was completed in 2003, allowing all diseases to be analyzed at the genetic level. Gene therapy has attracted attention as a method for treating genetic diseases such as cancer and familial Alzheimer's disease. The characteristic of gene therapy is expected to be applied to the ability to radically treat diseases at the genetic level.

Recently, the most actively studied gene therapy is the CRISPR system. Announced in 2012, CRISPR-Cas9[1] won the Nobel Prize as a tool for easily cleaving genomic DNA. The CRISPR system is improving day by day, allowing CRISPR-Cas13 to specifically cleave RNA. In the fields of genetic engineering and gene therapy, cutting tools are indispensable for utilizing nucleic acids. However, the CRISPR system has major problems such as off-target effect. I was interested in editing nucleic acids with simpler and easier operations, and decided to research new nucleic acid editing tools.

## Results and Discussion

[Chapter 2]

We aimed to develop a method for manipulating DNAzyme activity with light spatiotemporally using $^{CNV}$K [2]. A mask strand complementary to itself was extended from the 3' end of the DNAzyme, and $^{CNV}$K was introduced into a part of the mask strand. The DNAzyme in which the mask strand and the substrate region were photo-cross-linking succeeded in completely inhibiting the invasion of the substrate strand by covalent bonding. It was also clarified that the covalent bond by photo-cross-linking can completely block the invasion of exonuclease, so that it also has resistance to enzymatic degradation.

When $^{CNV}$K was photolyzed by irradiating the photo-cross-linked DNAzyme with photo at 312 nm, the substrate chain was invaded to the catalyst core, and 38% RNA cleavage was confirmed in a reaction of 10 equal doses and 15 min. As described above, $^{CNV}$K-introduced DNAzyme is expected to be applied to nucleic acid therapy because it is easy and accurate to switch the activity and has enzyme resistance[3].

[Chapter 3]

We have reported thermal irreversible DDI by antigene probe with $^{CNV}$K and self-photo-cross-linking inhibitor (5-cyanouracil, $^{CN}$U) [4]. In this chapter, we newly examined inhibitors (Spacer, dSpacer) that can suppress self-photo-cross-linking more than $^{CN}$U, and aimed to improve DDI efficiency. The self-photo-cross-link inhibition rates of Spacer (S) and dSpacer (dS) were obtained as 89.1% and 86.0%, respectively, which were (79.0%) higher than those of $^{CN}$U. However, the DDI photo-cross-linking rates of Spacer and dSpacer were calculated to be 4.1% and 3.5%, respectively, which were lower than the photo-cross-linking rate (26.1%) of $^{CN}$U. DDI photo-cross-linking is thought to consist of several equilibrium reactions, and the thermodynamic parameters of the probe were newly measured. The Tm values of each inhibitor were calculated as 37.3 ℃ for T, 33.5 ℃ for $^{CN}$U, 24.9 ℃ for dS, and 25.2 ℃ for S. Comparing these results, it was found that DDI photo-cross-linking consists of a balance between self-photo-cross-linking between probes and stability with target DNA [5].

[Chapter 4]

DNA carries the genetic information of living organisms and has a double helix structure that maintains a very stable state. In addition, the longer the DNA, the more stable it becomes, and it may form complex secondary or tertiary structures. Antigene methods such as PNA and LNA are verified with a model sequence of about 200-400 mer for genomic DNA editing.

We prepared a sequence obtained by extending the model sequence used in Chapter 3 to 400 mer and verified DDI photo-cross-linking. An antigene probe with Cy3 (11bp-5nt) confirmed a band derived from the photo-cross-linker with the target sequence. When 11bp-5nt was used as the DDI probe, a maximum photocrosslinking rate of 78% with the target DNA was observed. The optimal reaction conditions were 1 min, 1 h, and 100 eq. for light irradiation time, incubation time, and equivalent dose, respectively.

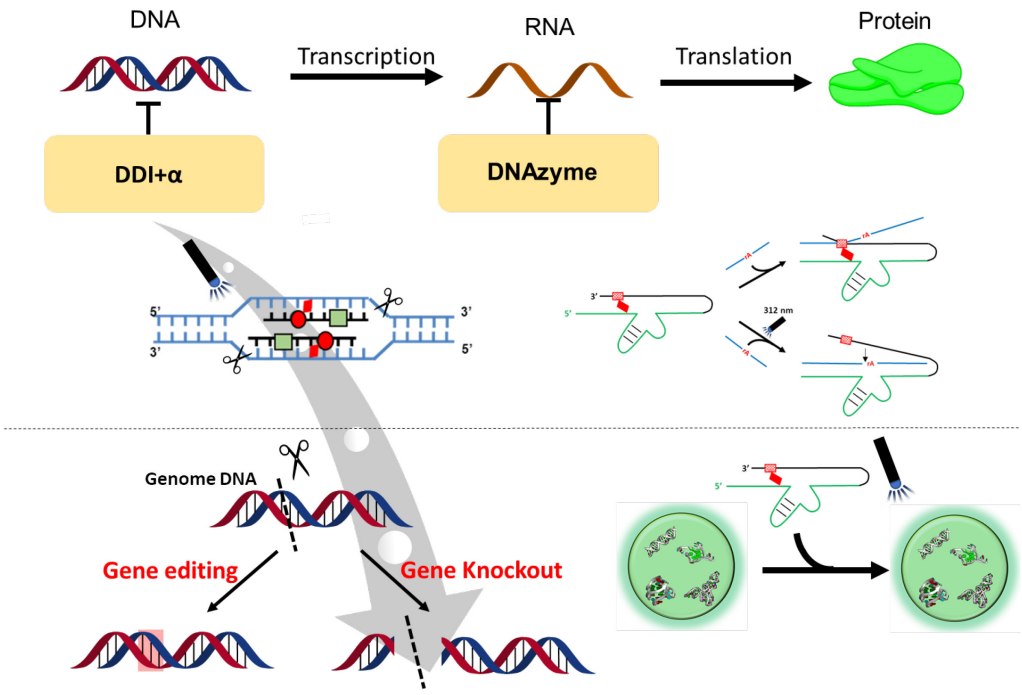


Figure.　Overview of future work using ribozymes and DDI

## Keywords

Genome editing, Photo-cross-linking, 3-cyanovinylcarbazole, DNAzyme, Double duplex invasion

**論文審査の結果の要旨**

CRISPR-Cas9 技術に代表される様にゲノム操作のための新しい遺伝子工学的手法の開発は、幹細胞工学、遺伝子治療、組織や動物の疾患モデル、遺伝子組み換え植物の技術など幅広い用途・分野において新産業を産み出す力に直結している。本論文はゲノム操作の一つとして考えられる核酸編集を指向し、超高速光架橋を用いた DNA および RNA 操作法の開発をおこなったものであり、以下の点で有用かつ独創的な内容であった。

RNA を分解する酵素として RNase が知られているが、RNase を用いる際、酵素ゆえに至適 pH，至適温度，至適塩強度の条件下で用いる必要があった。これら制約条件から解放され、より汎用性が高くなる例えば時空間制御可能な人工 RNA 分解酵素の設計と開発をおこなった。RNA を配列選択的に分解する人工 RNA 分解酵素として知られている DNAzyme 中に研究室で開発済みの光架橋性シアノビニルカルバゾールヌクレオシド（$^{CNV}$K）を埋め込み、可逆的 DNA 光クロスリンク能を有する人工 RNA 分解酵素を作成した。この人工 RNA 分解酵素はあらかじめ光架橋させておくことで RNA 分解を完全に抑制することができ、光スイッチによって OFF-ON 制御可能であることを見出した。また架橋しておくことで DNA 分解酵素に対して耐性を持っていることも併せて見出した。

次に、酵素特有の制約条件の影響を受けずに時空間制御可能な DNA 2 本鎖を配列選択的に切断する人工制限酵素の設計と開発をおこなった。その際、基質となる DNA 2 本鎖が非常に安定な 2 重らせん構造を有することから、オリゴ核酸(ODN)が DNA 2 本鎖に対して相互作用しようとしてもすぐ押し出されてしまうため、DNA 2 本鎖に対して配列選択的に相互作用する人工核酸の報告例が殆どなかった。そこで、DNA 2 本鎖に対

して $^{CNV}$K を埋め込んだ人工核酸プローブをそれぞれ片側の DNA 鎖に同時に相互作用させることで double duplex invasion (DDI)という安定な構造を構築できるのではと考えた。まず、$^{CNV}$K の架橋位置に自己架橋抑制素子となる塩基を 4 種類(チミン (T)、5-cyanouracil ($^{CN}$U), Spacer, dSpacer)検討し、$^{CN}$U が DDI 構築の際に副反応として考えられる光応答性プローブ同士の架橋反応を最も抑制することを見出した。次にこの $^{CNV}$K と $^{CN}$U を併せもつプローブを 400 塩基対の長鎖 ODN に対して相互作用させ 385 nm 光照射を 1 秒おこなったところ、70%以上の高収率で DDI 構造が構築できることを見出した。

以上、本論文は、時空間制御可能な核酸編集を指向した新規核酸類操作法の開発に関するもので、学術的に貢献するところが大きい。よって博士（マテリアルサイエンス）の学位論文として十分価値あるものと認めた。