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種別(和文)	論文要旨
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（博士課程）
Doctoral Program

論 文 要 旨

THESIS SUMMARY

系・コース： Department of, Graduate major in	生命理工学 系 生命理工学 コース	申請学位（専攻分野）： Academic Degree Requested	博士 (Science) Doctor of
学生氏名： Student's Name	Yaoyao DU	審査員主査： Chief Examiner	湯浅 英哉

要旨（英文 800 語程度）
Thesis Summary (approx.800 English Words)

This doctoral thesis is titled "Development of a site-selective photooxidation method to target genome DNA by biphenyl photosensitizer – PNA conjugates" and comprises five chapters. The aim of this study is to develop an alternative photo-knockout approach to overcome the limitations of unnecessary immune response caused by large nuclease in CRISPR/Cas9 system. To achieve this goal, a small-molecule method using nitrobiphenyl photosensitizer (NBP)-peptide nucleic acids (PNA) conjugates is proposed to produce targeted break in genome DNA via site-selective photooxidation of guanine (G).

Chapter 1 is "Introduction and Background", describing the abstract, the background information and design of this thesis work. Firstly, to address the unwanted immune responses toward nuclease, the strategy of site-selectively inducing single-strand breaks (SSBs) in DNA through the repairment of oxidative damage is proposed. Then the basis of this study is briefly summarized, including the development of a small-size type II photosensitizer (PS) NBP and distance-dependent oxidation of G by NBP-DNA conjugates. Finally, the design of this thesis work is outlined. A novel NBP-PNA conjugate for photo-induced DNA cleavage in target double-strand DNA (dsDNA) is designed, whose specificity relies on two factors: the site-specific invasion of PNA into dsDNA and selective oxidation of G by singlet oxygen ($^1O_2$) generated from NBP.

Chapter 2 is "Synthesis and Characterization of NBP-PNA Oligomers". The NBP group is attached to the α-methyl position of Thymine via an ethylene linker, and this novel NBP-Thymine-PNA monomer is synthesized through an 8-step synthesis routine. Then PNA oligomers, each consisting of 15 bases and incorporating a hydrophilic lysine at the N-terminus, are synthesized using optimized Fmoc-based solid-phase synthesis. The thermal denaturation measurements (T_m) show modest differences between NBP-conjugated-PNA/DNA (44.4 °C) and unmodified PNA/DNA duplexes (46.1 °C), and their CD spectra have two similar absorption bands (220 and 260-270 nm), suggesting that incorporation of NBP in PNA does not cause any significant structural deviations from the right-handed helix.

Chapter 3 is "Photooxidation of ssDNA and ds DNA by NBP-PNAs". Firstly, the $^1O_2$ production ability of NBP-PNA is confirmed to be at the same level with NBP-DNA by monitoring the consumption of furfuryl alcohol (FA) via HPLC. Then, quantitative analysis by RP-HPLC reveals that the efficiencies of G photooxidation in NBP-PNA/DNA duplexes are 4.2%, 2.5% and 2.4%, significantly lower than those in corresponding NBP-DNA/DNA duplexes (62%, 6.7% and 9.4%), respectively. In addition, the G photooxidation efficiency in unmodified-PNA/DNA by an external type II PS, xylitol-NBP, is 28.9%, extremely lower than that of dsDNA 72.5%. These results suggest that PNA complexed with DNA suppresses G oxidation. Subsequently, to explain this observation, two hypotheses are presented: the first is the base stacking within PNA/DNA duplex reduced accessibility to G, which is supported by an additional experiments that the placements of the abasic site in PNA strand around G, resulting in much space around G, yield higher photooxidation

efficiencies (4.5%, 9.2%, 3.2%) than the fully matched NBP-PNA/DNA (2.5%); the second suggests that $^1O_2$ produced from NBP-PNA is physically quenched during diffusion along the PNA/DNA backbone. Finally, gel electrophoresis experiments demonstrat that when complexed with the sticky end of a dsDNA, NBP-PNA conjugate is able to photooxidize G only in the dsDNA region upon irradiation, while the NBP-DNA can also photooxidize G in the sticky end region. Thus, these results suggest that NBP-PNA conjugate is a potential tool to site-selectively induce oxidation of G in dsDNA when properly designed.

Chapter 4 is "End-invasion and Photooxidation of dsDNA by NBP-PNA". Firstly, the non-denaturing gel electrophoresis exhibits the formation of NBP-PNA/dsDNA end-invasion complex when stoichiometric ratio of PNA to dsDNA is 80 or 100, indicating the addition of NBP hardly hamper PNA from invading dsDNA. However, when a necessary large amount of PNA is incubated with dsDNA for invasion, the self-aggregator of hydrophobic PNA oligomers would non-specifically interact with dsDNA and form $(PNA)_n$-DNA aggregation which tends to be adsorbed onto hydrophobic surface. What's more, such $(PNA)_n$-dsDNA aggregation might have inhibited the photooxidation as no cleavage products are observed in NBP-PNA/dsDNA invasion solution with irradiation and treatment by Fpg or hot piperidine. In addition, external xylitol-NBP can photooxidize unmodified-PNA/dsDNA (1:1) invasion complex and produce two cleavage products, the cleavage efficiencies are determined to be 12.9% and 8.6% with hot piperidine treatment. With the use of larger amount PNA invasion sample with unmodified-PNA/dsDNA (100:1), however, the cleavage efficiencies decrease to 0.9% and 1.2%, respectively.

Chapter 5 is titled "Summary" which concludes this thesis and discusses the further development of NBP-PNA conjugates. Overall, it is found that the PNA complexed with DNA suppresses G oxidation, and NBP-PNA conjugate bound to the sticky end of a dsDNA can site-selectively photooxidize G only in the dsDNA region. Additionally, it is necessary to increase the invasion effectiveness of NBP-PNA in further study to avoid the formation of $(PNA)_n$-DNA aggregation. Ultimately, this site-specific photooxidation approach using NBP-PNA conjugates could expand the toolkit for precise gene editing.