Photo-cross-linking oligonucleotides for bioorthogonal regulation of gene expression

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Regulation of gene expression in sequence-specific manner in biological systems without damaging the biological activities are much important issues in medical, biological and molecular biological fields. Photo-cross-linking oligonucleotides bearing photo-induced reactive moieties are powerful tools because the photo-induced cross-linking reaction can be spatiotemporally and bioorthogonally controlled via ultraviolet (UV) irradiation. In this thesis, I develop novel photo-cross-linking oligonucleotides and investigate their properties *in vitro* and in cells.

Chapter 1 concerns the development of novel photo-cross-linking oligonucleotides (PBA-ODNs) bearing a photoresponsive α-bromoaldehyde as a photoreactive group to investigate the kinetics of the photo-cross-linking reactions with target molecules. The photo-cross-linking oligonucleotides reacted with target nucleotides having an adenine or a cytosine at the frontal position of the reactive aldehyde with much higher kinetic rates than photoresponsive α-chloroaldehyde-conjugated oligonucleotides reacted.

Chapter 2 concerns the inhibition effects of photo-cross-linking oligonucleotides (PXA-ORNs) bearing a photoresponsive α-haloaldehyde on mRNA translation. Using HeLa cells expressing GFP, PXA-ORNs effectively suppressed the fluorescent intensity of GFP under UV-irradiation conditions. These results suggest that PXA-ORNs could be activated in cells and inhibit the mRNA translation in sequence-specific manner.

Chapter 3 concerns the development of novel photo-cross-lining oligonucleotides (^{pro}PCA-ODNs) bearing a caged α-chloroaldehyde group conjugated to a 2-methylpropanediyl backbone (^{pro}PCA) in the middle of the strand for selective reactions with RNAs having point mutations. Photo-cross-linking studies of ^{pro}PCA-ODNs with complementary oligoribonucleotides (ORNs) revealed that ^{pro}PCA-ODNs reacts efficiently and selectively with the target ORNs that have an adenosine or cytidine residue at a frontal position of the ^{pro}PCA residue without adverse effects of bases adjacent to the mutation site.

Chapter 4 concerns the development of novel photo-cross-linking oligonucleotides (X-ODNs) bearing an adenosine derivative with a 3-trifluoromethylphenyldiazirine at 2'-position for rapid interstrand cross-linking formations. Photo-cross-linking studies revealed that the X-ODNs selectively cross-linked with the oligo-DNAs (and not with the oligo-RNAs), and 5 min of UV irradiation was sufficient for the photo-crosslinking reactions.