Site-Directed Mutagenesis Studies Using Photo-Chemical Nucleic Acid Editing

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Introduction

Nucleic acid chemistry and its biological applications have a great scope in the development of futuristic drugs and cure for many diseases. Enzymatic methods for the nucleic acid manipulation have proven to be very useful *in vitro* but they have their disadvantages in the *in vivo* applications.[1] Therefore, chemical methods to edit nucleic acids were developed but the drawbacks of those chemical methods are numerous.[2]

Thus, to overcome these problems, photochemical methods to edit the nucleic acids have been devised which utilize single base modified nucleo-base to specifically target a desired sequence of DNA/RNA and edit that sequence at a single point. Fujmoto's group has discovered a novel compound, 3-Cyanovinylcarbazole, which can be easily incorporated as a nucleo-base and upon irradiation of 366nm radiation, forms a crosslink with the pyrimidine and lead to deamination to afford the transformation of the cytosine to uracil. The crosslink is photo-reversible and can be easily converted back by 312nm irradiation.[3-6]

The experiments have already shown success in the small nucleic acid sequences.[7-8] Moreover, the feasibility of the photo-crosslinking reaction using 3-Cyanovinylcarbazole *in vivo* has also been reported.[9]

Major drawback of this method is that the deamination step takes place at 90ºC, which is not a feasible condition for the *in vivo* applications. Therefore, in this research, the focus on development new method, such that, the deamination, which takes 200 years in physiological conditions without any external factor, can be carried out at 37ºC, i.e. physiological conditions in shorter time.

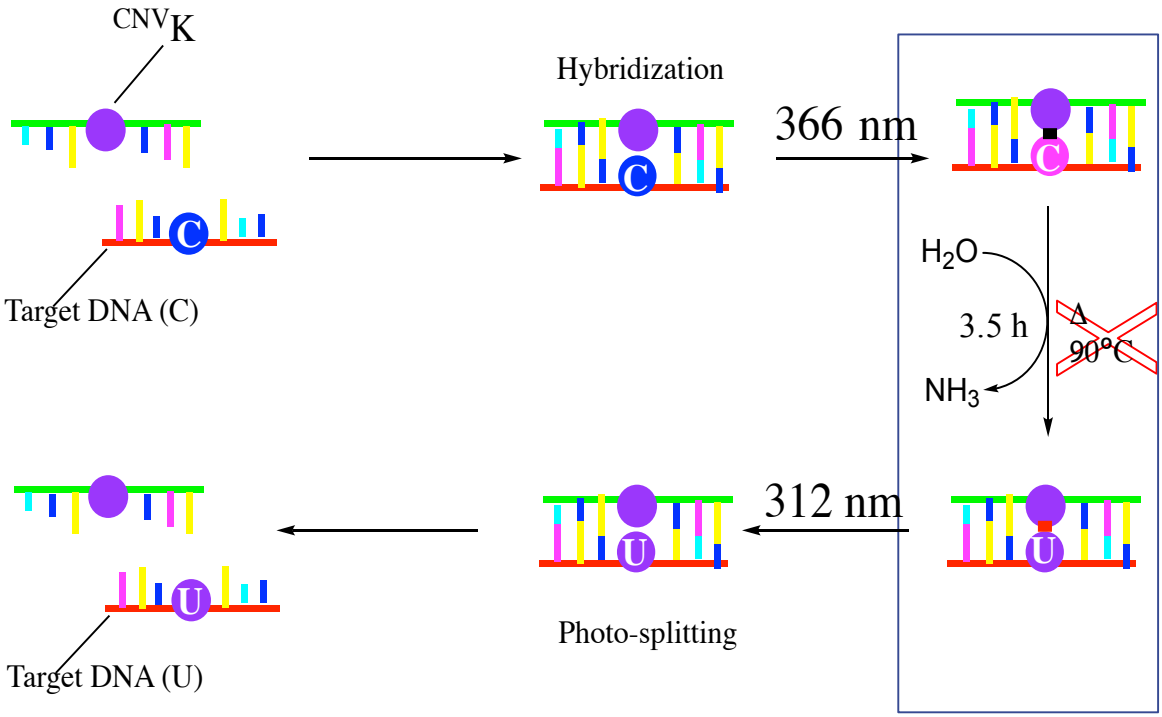

Figure 1: Schematic overview of photo-chemical DNA editing.

Results and Discussion

Chapter 2: The focus was to find the best counter-base of cytosine, based on hydrogen bonding, for photo-chemical site-directed mutagenesis using 3-cyanovinylcarbazole as the photo-active nucleoside which can crosslink with the target cytosine to afford cytosine to uracil transformation at physiological conditions. Different counter bases like guanine (G), inosine (I), 2-aminopurine (P), nebularine (R), and 5-nitroindole (N) were used to find the best counter base. Among all the bases, it was found that P, R, and N are not suitable counter base for photo-chemical cytosine to uracil transformation as when using these bases, no deamination reaction takes place. While in case of G, the deamination reaction is very slow and only 5% conversion is observed in 72h reaction time. Thus, the best counter base among the bases was found to be inosine which gives 35-40% in 72h reaction time at 37ºC having the optimal hydrogen bonding pattern before and after photo-cross-linking.

Chapter 3: In this chapter, the role of hydrophilicity and polarity of photo-cross-linker was discussed. Various derivatives of vinyl carbazole, like 3-cyanovinyl carbazole (^{CNV}K), 3-amidovinylcarbazole (^{NH2V}K), 3-methoxyvinylcarbazole (^{OMeV}K), and 3-carboxylvinylcarbazole (^{OHV}K), were used for studying the micro-environment around the

target cytosine crosslinked to photo-cross-linker during the deamination of cytosine. It was discovered that the hydrophilicity and polarity of the photo-cross-linker plays a crucial role in the deamination of cytosine to uracil via photo-cross-linking. ^{OMeV}K having the least hydrophilicity gave the least rate of reaction for the deamination reaction at varying temperature (90, 70, 50, and 37 ºC) while the highest reaction rate was observed with ^{OHV}K, which is most polar among the cross-linkers based on the polarity index (Log P). Thus, hydrophilicity and polarity around target cytosine are deciding factors in case of deamination reaction of cytosine via photo-cross-linking.

Chapter 4: Based on the findings of chapter 1 and 2, the overall micro-environment around the target cytosine for the mutation of cytosine to uracil via vinylcarbazole based photo-cross-linking was studied. A combination of counter bases (guanine (G), inosine (I), and cytosine (C)) and photo-cross-linkers (^{CNV}K, ^{NH2V}K, and ^{OHV}K) were used in the ODN to study the best match for acceleration of deamination of cytosine to uracil at physiological conditions. It turned out that the best combination of counter base and photo-cross-linker is inosine and ^{OHV}K which could give ~70% conversion of cytosine to uracil in 7 days at physiological conditions, which could be extended to ~90% in 20 days. The micro-environment around cytosine, including hydrogen bonding, hydrophilicity, and polarity of counter base and photo-cross-linker are key players for the photo-cross-link assisted deamination of cytosine to uracil.

Chapter 5: Based on the previous chapters we realized that the micro-environment around the target cytosine is deciding factor for rate of cytosine to uracil conversion via photo-cross-linking. Although, the reaction rate is very rather slow at physiological conditions even when inosine is counter base and ^{OHV}K is photo-cross-linker. Thus, a different approach to accelerate the rate of deamination reaction was used in which the ODN containing photo-cross-linker was divided into two parts between the counter base and photo-cross-linker. The adjoining part was modified with phosphate group at the terminal of counter base to increase the hydrophilicity near the cytosine. It was observed that upon the phosphate group modification near cytosine, ~100% conversion of cytosine to uracil was observed in just 24 h. Furthermore, we removed the ODN with counter base and modified the photo-cross-linker end with phosphate group to study the rate of reaction without hydrogen bonding and high hydrophilicity. It was found that the rate of reaction increased multifold with the modification giving ~100% conversion from cytosine to uracil in 3h at physiological conditions.

These results indicate that the deamination of cytosine to uracil is feasible at physiological conditions and heating to very high temperature is no more necessary to achieve the site-directed mutagenesis via photo-cross-linked cytosine. This has opened vast opportunities to use this enzyme free system in the biological samples at reduced cost and complexity to afford specific and site-directed cytosine to uracil conversions for the treatment of various genetic disorders like Leigh's syndrome.

Future Prospects

In this study, I have developed a refined way to carry out site-directed mutagenesis using enzyme free photo-chemical methods at physiological conditions. This technique has wide applications in the field of anti-sense technology, RNAi, RNA/DNA editing in cell for genome engineering to eradicate certain genetic disorders arising due to single T→C point mutations.

References

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