## Effects of Hydrogen Bonding and Vinylcarbazole Derivatives on 3-Cyanovinylcarbazole Mediated Photo-Cross-Linking Induced Cytosine Deamination

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Abstract : Site-directed mutagenesis is a renowned technique to introduce specific mutations in the genome. To achieve sitedirected mutagenesis, many chemical and enzymatic approaches have been reported in the past like disulphite induced genome editing, CRISPR-Cas9, TALEN etc. The chemical methods are invasive whereas the enzymatic approaches are timeconsuming and expensive. Most of these techniques are unusable in the cellular application due to their toxicity and other limitations. Photo-chemical cytosine deamination, introduced in 2010, is one of the major technique for enzyme-free singlepoint mutation of cytosine to uracil in DNA and RNA, wherein, 3-cyanovinylcarbazole nucleoside (CNVK) containing oligodeoxyribonucleotide (ODN) having CNVK at -1 position to that of target cytosine is reversibly crosslinked to target DNA strand using 366 nm and then incubated at 90°C to accommodate deamination. This technique is superior to enzymatic methods of site-directed mutagenesis but has a disadvantage that it requires the use of high temperature for the deamination step which restricts its applicability in the in vivo applications. This study has been focused on improving the technique by reducing the temperature required for deamination. Firstly, the photo-cross-linker, CNVK has been modified by replacing cyano group attached to vinyl group with methyl ester (OMeVK), amide (NH2VK), and carboxylic acid (OHVK) to observe the acceleration in the deamination of target cytosine cross-linked to vinylcarbazole derivative. Among the derivatives, OHVK has shown 2 times acceleration in deamination reaction as compared to CNVK, while the other two derivatives have shown deceleration towards deamination reaction. The trend of rate of deamination reaction follows the same order as that of hydrophilicity of the vinylcarbazole derivatives. OHVK being most hydrophilic has shown highest acceleration while OMeVK is least hydrophilic has proven to be least active for deamination. Secondly, in the related study, the counter-base of the target cytosine, guanine has been replaced by inosine, 2-aminopurine, nebularine, and 5-nitroindole having distinct hydrogen bonding patterns with target cytosine. Among the ODNs with these counter bases, ODN with inosine has shown 12 fold acceleration towards deamination of cytosine cross-linked to CNVK at physiological conditions as compared to guanosine. Whereas, when 2aminopurine, nebularine, and 5-nitroindole were used, no deamination reaction took place. It can be concluded that inosine has potential to be used as the counter base of target cytosine for the CNVK mediated photo-cross-linking induced deamination of cytosine. The increase in rate of deamination reaction has been attributed to pattern and number of hydrogen bonding between the cytosine and counter base. One of the important factor is presence of hydrogen bond between exo-cyclic amino group of cytosine and the counter base. These results will be useful for development of more efficient technique for site-directed mutagenesis for  $C \rightarrow U$  transformations in the DNA/RNA which might be used in the living system for treatment of various genetic disorders and genome engineering for making designer and non-native proteins.

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Keywords : C to U transformation, DNA editing, genome engineering, ultra-fast photo-cross-linking

Conference Title : ICNA 2017 : International Conference on Nucleic Acids

Conference Location : Venice, Italy

Conference Dates : June 21-22, 2017