Nucleotide insertion opposite a cyclobutane pyrimidine analogue caused from photoligation by a replicative DNA polymerase

Masayuki Ogino¹, Daisuke Okamura¹, Yoshinaga Yoshimura² and Kenzo Fujimoto¹,²
¹The School of Materials Science, Japan Advanced Institute of Science and Technology, 1-1 Asahidai, Nomi, Ishikawa 923-1292, Japan and ²PRESTO- Japan Science and Technology Agency

ABSTRACT

We previously reported an efficient and reversible template-directed photoligation using 5-carboxyvinyl-2'-deoxyuridine (5-CVU)-containing ODN at the 5’-terminal. This method forms d(T-5-CVU) as a cyclobutane pyrimidine dimer (CPD) analogue between 3'-terminal thymidine and 5'-terminal 5-CVU of two oligodeoxynucleotides (ODNs). In this study, we performed PCR using a DNA template containing d(T-5-CVU). And then, we disclosed that two adenines were incorporated opposite the d(T-5-CVU).

INTRODUCTION

Nucleic acid templated syntheses prior to the current decade predominantly used DNA or RNA templates to mediate ligation reactions that generate oligomers of DNA, RNA or structural analogues of nucleic acids.¹ In addition to analogues of the phosphoribose backbone, products that mimic the structure of stacked nucleic acid aromatic bases have also been generated by DNA-templated synthesis.² We previously described a reversible template-directed photoligation mediated by [2 + 2] cycloaddition that was modeled on the structure of cyclobutane pyrimidine dimer (CPD) between 3'-terminal thymidine and 5'-terminal 5-carboxy-2'-deoxyuridine (Figure 1).³ While non-enzymatic ligation methods may offer some advantages, one limitation of the photoligation strategy relative to enzymatic methods is the fact that the CPD analogue as the ligated structures differs from that of the natural DNA junction, which is likely to interfere with further manipulation (such as amplification). However, in vitro analyses have shown that some DNA polymerases can copy distorted DNA templates containing a UV-induced lesion.⁴ In this study, we examined some DNA polymerases which can accept the DNA template containing d(T-5-CVU) during the PCR, and we found that Ex Taq DNA polymerase can read a template DNA containing d(T-5-CVU). Additionally, we determined that two adenines were incorporated opposite d(T-5-CVU).

RESULTS AND DISCUSSION

⁵-CVU was prepared according to the method reported previously.⁵ ODN containing ⁵-CVU, 5'-d(C⁵UGGAGCTGCGTTGGCGGCTCCCGT)-3' (ODN 1) was synthesized using phosphoramidite of ⁵-CVU according to conventional DNA synthesis. ODN 1 was characterized by the nucleoside composition and MALDI-TOF MS (caled 7714.01 for [M + H]+, found 7714.29).

We determined feasibility of the template-directed photoligation via ODN 1 (step 1 in Scheme 1). When ODN 1 and biotinylated ODN 2 (55 mer) were irradiated at 366 nm for 1 h in the presence of template ODN 3, we observed

![Scheme 1 Strategy for the synthesis of template ODN for PCR.](https://academic.oup.com/nass/article-abstract/50/1/125/1341173/83x788)

Figure 1 Structure of thymine dimer (a), and d(T-5-CVU) photoadduct (b).

![Figure 1 Structure of thymine dimer (a), and d(T-5-CVU) photoadduct (b).](https://academic.oup.com/nass/article-abstract/50/1/125/1341173/83x788)
the appearance of the peak of ligated ODN 4 (80 mer) as determined by capillary gel electrophoresis (CGE) with the disappearance of ODN 1 and ODN 2.

The ligated ODN 4/ODN 3 hybrids were subsequently immobilized on streptavidin-linked magnetic beads (step 2 in Scheme 1). The ligated ODN 4 was purified from unreacted ODN 2 and template ODN 3 on streptavidin-linked magnetic beads by washing twice with 10 mM tris-HCl (pH 7.5), 1 mM EDTA and 1 M NaCl (2 min, 80 °C) and eluting under 10 mM EDTA and 95% formamide (5 min, 60 °C) (step 3, 4 in Scheme 1).

We examined several DNA polymerases which were expected to accept the ODN 4 containing d(5'-3')U during the PCR. Figure 2 shows the results of the PCR with the substrate ODN 4. Ex Taq DNA polymerase could accept ODN 4 and form 80 bp PCR products. And then, we investigated the sequence of the elongation products. The sequencing results showed that the PCR products contain two adenines opposite d(5'-3')U, in contrast to the cDNA of two thymidines at that position.

Figure 2 Microchip electrophoresis of the PCR of ODN 4 with DNA polymerase. Lane 1: ODN 2 as PCR template with Ex Taq; Lane 2: 80 mer natural DNA as PCR template with Ex Taq; Lane 3: ODN 4 as PCR template with Ex Taq.

CONCLUSION

The present study demonstrated that d(5'-3')U-containing DNA were substrates for Ex Taq DNA polymerase yielding the corresponding PCR products. Additionally, we found that two adenines were preferentially inserted opposite d(5'-3')U. Employing these methods, we are currently investigating a new CPD analogue that was synthesized from other photosensitive nucleosides.

REFERENCES


5. The reaction mixture (total volume 60 μL) containing ODN 1 and ODN 2 (each 10 μM strand concentration) in the presence of template ODN 3 (10 μM strand concentration) in sodium cacodylate buffer (50 mM, pH 7.0) and sodium chloride (100 mM) was irradiated with a 25 W transilluminator (366 nm) at 0 °C for 1 h.

6. Realtime PCR was set up using a 25 μL volume, with 10 nM ODN 4, 10 mM Tris-HCl, 50 mM KCl, 2 mM MgCl₂, each dNTP 0.4 mM, 0.3 μM primer pair, 1 x SYBER Green and 2.5 unit ExTaq DNA polymerase. The PCR consisted of denaturation at 94 °C for 10 min, and 50 cycles of 94 °C for 10 s, 60 °C for 20 s and 72 °C for 20 s, and was followed by a final extension of 72 °C for 10 min.