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Template-directed reversible photocircularization of DNA via 5-vinyldeoxycytidine

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Abstract

An efficient and reversible template-directed photoligation of oligodeoxynucleotides (ODNs) using 5-vinyldeoxycytidine (^VC) was described. When ODN containing ^VC at the 5' end was photoirradiated with ODN containing thymine at the 3' end in the presence of a template ODN, an efficient photoligation was observed. Reversible photopadlocking of a circular DNA by the photocircularization using ^VC was demonstrated. © 2000 Elsevier Science Ltd. All rights reserved.

Although many methods are known for template-directed chemical ligation of ODN, a few methods for photoinduced chemical ligation has been demonstrated.¹ Recently, we disclosed an efficient and reversible template-directed photoligation of oligodeoxynucleotides (ODNs) using 5-vinyldeoxyuridine (^VU)-containing ODN at the 5' end.² However, due to the limitation of ^VU which is only applicable to adenine as a counter base in a duplex, a different base that contains a photoreactive vinyl group is required. We now report a reversible photoligation of 5-vinyl-deoxycytidine (^VC)-containing ODN at the 5' end with ODN containing thymine at the 3' end.³ By the use of ^VC-containing ODNs, we demonstrated a reversible formation of catenated DNA via template-directed photocircularization.



Phosphoroamidite building block 4^4 for the synthesis of ^VC-containing ODNs was prepared from 5'-DMTr-deoxyvinyluridine 3'-phosphoramidite 3.5 ^VC-containing ODNs were synthesized according to the standard phosphoramidite chemistry using 4, coupled with a post-modification

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procedure of ammonia treatment as reported previously⁶ (Scheme 1). Incorporation of ^VC into ODN was confirmed by enzymatic digestion and ESI-TOF MS of the resulting ^VC-containing ODNs.⁷



Scheme 1. (a) DMTrCl, NEt₃, pyridine, 18 h, 73%; (b) P(N-*i*Pr)₂O(CH)₂)₂CN, tetrazole, CH₃CN, 1 h, 98%; (c) 1,2,4-triazole, POCl₃, NEt₃, CH₃CN, 3 h, 95%

When ³²P-5'-end-labeled d(TGTGCT) (ODN **5**) and d(^VCGCGTG) (ODN **6**a) were irradiated at 366 nm in the presence of template ODN **7a** (Scheme 2), the expected ligated 12 mer ODN **8a** was produced in 93% yield as determined by densitometric assay of the PAGE (Fig. 1, lane 2).⁸ Quantitative formation of starting ODN **5** was observed by further irradiation of lane 2 at 302 nm (Fig. 1, lane 3). HPLC analysis of the photoirradiated mixture of ODN **5** and ODN **6a** with template ODN **7a** confirmed a clean and efficient formation of ligated ODN **8a** with concomitant disappearance of ODN **5** and ODN **6a**. The ESI-TOF MS indicated that ODN **8a** is a ligated product between ODN **5** and ODN **6a**.⁹ Enzymatic digestion of isolated ODN **8a** indicated the formation of dG, dT and dC in a ratio of 5:3:2 together with a dT-^VC photoadduct.¹⁰ These results indicated that ODN **8a** is a photoligated product via the photolinking of 3'-terminal thymine and 5'-terminal ^VC. The reversibility of the photoligation of ODN **8a** (X = ^VC) and ODN **8b** (X = ^VU) upon 302 nm irradiation was compared under identical conditions. As shown in Fig. 1, ^VC-containing ODN is far superior to ^VU-containing ODN for the photosplitting of ligated ODNs (lane 3 versus lane 6).



Scheme 2.



Figure 1. Autoradiogram of a denaturing polyacrylamide gel electrophoresis of the photoreaction of ODN 6a,b and $^{32}P-5'$ -end-labeled ODN 5 in the presence of template ODN 7a,b. Lane 1: ODN 5+ODN 6a/ODN 7a, without photo-irradiation; lane 2: irradiation of lane 1 at 366 nm, 3 h (93%); lane 3: irradiation of lane 2 at 302 nm, 1 h (quant.); lane 4: ODN 5+ODN 6b/ODN 7b, without photoirradiation; lane 5: irradiation of lane 4 at 366 nm, 3 h (98%); lane 6: irradiation of lane 5 at 302 nm, 1 h (30%)

Reversible photopadlocking of circular plasmid DNA by the photocircularization using ^VC-containing ODN was examined.^{1d} A long 60 mer ODN **9** containing two sites that are complementary to the 20 nucleotide section of plasmid M13mp18 DNA was synthesized by standard automated DNA synthesis (Fig. 2). On the gel of the photoirradiated ³²P-5'-end-labeled ODN **9** in the presence of M13mp18 DNA, a slower moving band which has the same mobility as the band produced by T4 DNA ligase treatment appeared in 25% yield (Fig. 3, lane 3).¹¹ Further irradiation of lane 3 at 302 nm resulted in a complete reversion to original linear ODN **9** (lane 4). As shown in lane 5, photopadlocked DNA was observed again by 366 nm irradiation, indicating the reversibility of the photocircularization process. To our knowledge, this is the first demonstration of the reversible DNA photocircularization that produces DNA catenane, although irreversible padlocking of plasmids has been reported in several systems.^{12,1d}

In conclusion, we have demonstrated a reversible photoligation of ODNs containing thymine at the 3' end with ODNs containing ^{V}C at the 5' end. The ^{V}C mediated reversible photoligation was very useful for padlocking circular DNA to produce DNA catenane.



Figure 2. Plasmid DNA and ODN used in this study. The target sequence was located from position 6054 to 6073 in plasmid M13mp18. The sequence of 60 mer ODN was shown. 60 mer ODN **9** was hybridized to this plasmid at both 3' and 5' ends



Figure 3. Autoradiogram of 12% denaturing gel electrophoresis for the photoligation of ODN 9 in the presence of plasmid M13mp18. Lane 1: ODN 9; lane 2: ODN 9+M13mp18+T4 DNA ligase, rt, 3 h (20%); lane 3: ODN 9+M13mp18, irradiation at 366 nm, 3 h (25%); lane 4: irradiation of lane 3 at 302 nm, 30 min (quant); lane 5: irradiation of lane 4 at 366 nm 3 h (13%)

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- 3. ^VC: λ_{max} (water) 289 nm, ε 5,400 (ε at 366 nm, 13).
- 4. ³¹P NMR (121 MHz, CDCl₃): δ 149.811, 150.386 (diastereomers).
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- 7. ESI-TOFF MS: calcd. for ODN **6a** ($C_{60}H_{75}N_{23}O_{35}P_5$) (M–H⁻): 1832.35; found: 1832.32.
- The reaction mixture (total volume 10 μL) containing ODN 6 (7 μM, strand conc.), ODN 5 (ca. 2×10⁴ cpm, 0.1– 0.3 μM) and template ODN 7 (9 μM, strand conc.) in 50 mM sodium cacodylate buffer (pH 7.0) was irradiated with a transilluminator (366 nm or 302 nm) at 0°C.
- 9. ESI-TOFF MS: calcd. for ODN 8a (C₁₁₉H₁₅₁N₄₂O₇₂P₁₀) (M–H⁻): 3629.68; found: 3629.67.
- 10. ESI-TOFF MS: calcd. for dT-^VC photoadduct ($C_{20}H_{25}N_4O_{10}$) (M–H⁻): 481.16; found: 481.15.
- 11. The reaction mixture (total volume 10 μ L) containing M13mp18 DNA (90 nM, strand conc.) and ODN **9** (ca. 2×10⁴ cpm, 30 nM) in 50 mM sodium cacodylate buffer (pH 7.0) was irradiated similarly.⁸
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