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Photoinduced DNA end capping via N³-methyl-5-cyanovinyl-2’-deoxyuridine

Kenzo Fujimoto, Yoshinaga Yoshimura, Tadayoshi Ikemoto, Akio Nakazawa, Masayuki Hayashi and Isao Saito

A modified oligodeoxynucleotide (ODN) containing N³-methyl-5-cyanovinyl-2’-deoxyuridine reacts by photoirradiation at 366 nm with an adenine residue of a complementary template ODN to yield an end-capped ODN in 87% yield.

Since the double helical structure of DNA was first described by Watson and Crick in 1953, a wide variability of DNA conformations has been observed as non-ground state structures, such as hairpin-DNA, cruciform, Z-DNA and triple helix in nucleic acid. It has been difficult to study such unusual DNA conformations by biophysical analysis because of the narrow range of limited conditions under which they exist. Among these structures, the hairpin stem-loop structure has attracted interest because of its generality in palindromic sequences associated with the regulation of transcription and other biological functions. To overcome these problems, chemical probes for the trapping and stabilization of such hairpin structures have been developed to explore DNA conformations, dynamics and their biological roles.

Recently, we have reported efficient and reversible template-directed photoligations with ODNs containing 3’-terminal cytosine using 5-vinyl-2’-deoxyuridine (5’U) containing ODN at the 5’-terminal. A remarkable stacking between a vinyl residue of 5’U and 5’-pyrimidine within the same strand will be responsible for the efficient photoreaction in our template-directed DNA photoligation system via 5’U. We have now examined photochemical end capping, using N³-methyl-5-cyanovinyl-2’-deoxyuridine (MCVU) instead of 5’U, in which the more photoreactive vinyl group was incorporated. The photoreactive cyanovinyl group in MCVU was designed to stack effectively with a base in the opposite strand by an N³-methyl group substitution that allows stabilization of the syn orientation of MCVU and release from the Watson–Crick base pair (Figure 1). Herein we report the photochemical DNA end capping via MCVU instead of 5’U to generate the stabilized hairpin analogue at its end.

MCVU-containing ODN was synthesized according to the standard phosphoramidite chemistry on a DNA synthesizer. The phosphoramidite of MCVU was prepared in six steps from 5-iodo-2’-deoxyuridine as shown in Scheme 1. Incorporation of MCVU into ODN was confirmed by enzymatic digestion and MALDI–TOF–MS.

When 5’-d(MCVUGCGTG)-3’ ODN1(MCVU) was irradiated at 366 nm for 30 min in the presence of 5’-d(CACGCA)-3’ ODN1(A) (Scheme 2), ODN1(MCVU)-A was produced in 87% yield, as determined by HPLC analysis (Figure 2). MALDI–TOF–MS indicated that ODN1(MCVU)-A obtained by...
conformationally restricting with the T4 loop hairpin ODN, reflecting the effect of the linker resulted in an increase in thermal stability by 32° photochemically. It is also observed that end capping of ODN significantly increased melting temperature (ΔTm) of double-capped ODN ODN2(MCV(U-A)) was isolated and used in nucleolytic digestion for 30 min compared with quantitative degradation of starting ODN2(MCV(U)) (Figure 3, lane 4 and lane 7)14,15. No degradation of ODN2(MCV(U-A)) was observed in phosphodiesterase treatment for 24 h (Figure 3, lane 5). These results show that the end-capped ODN2(MCV(U-A)) increases significantly its stability in the biological medium and its possibility as a decoy DNA for directly targeting transcription factors and for globally controlling the expression of genes.16

In conclusion, we have synthesized MCV(U)-containing ODN as a probe for trapping and stabilizing the hairpin structure and demonstrated the photochemical end capping of ODN via MCV(U). This MCV(U)-mediated photochemical end capping may find application in the investigation of nucleic acid structure and function.

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Notes and references


5 MCV(U): λmax (water) 299 nm, ε 12,500 (at 366 nm, 85).

6 MALDI–TOF–MS: calcd. for ODN1(MCV(U)) [M-H+] 1873.30; found 1873.47.

7 The yield was calculated based on ODN1(A).

8 Each of the reaction mixtures containing ODN1(MCV(U)) (20 μM, strand concn) and ODN1(A) (20 μM, strand concn) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride in a Pyrex tube was irradiated with a 25 W transilluminator (366 nm, 200X, 200X, 00000

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5,700 µW/cm²) at 0 °C for 30 min. After irradiation, the progress of photoreaction was monitored by HPLC on a Chemcobond 5C18 ODS column (4.6 × 150 mm, elution with a solvent mixture of 50 mM ammonium formate, pH 7.0, linear gradient over 40 min from 3% to 10% acetonitrile at a flow rate 1.0 mL/min).

MALDI−TOF−MS: calcd. for ODN1(MCV-U-A) [(M−H)] 3633.52; found 3633.87.

MALDI−TOF−MS: calcd. for dA-dMCV photoadduct [(M+H)] 545.52; found 545.26.


The reaction mixture containing ODN2(MCV) (20 µM, strand concn) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride in a Pyrex tube was irradiated with a 25 W transilluminator (366 nm, 5,700 µW/cm²) at 0 °C for 3 h. Then, end-capped ODN2(MCV-U-A) was obtained from the isolated peak at 13.5 min from HPLC analysis. The progress of photoreaction was monitored by HPLC on a Chemcobond 5C18 ODS column (4.6 × 150 mm, elution with a solvent mixture of 50 mM ammonium formate, pH 7.0, linear gradient over 40 min from 3% to 12% acetonitrile at a flow rate 1.0 mL/min).

To a solution (0.5 mL) containing HPLC purified ODN2(MCV) (40 µM, strand concn) or ODN2(MCV-U-A) (40 µM, strand concn), snake venom phosphodiesterase (0.2 mL, 0.3 units/mL) was added and incubated at 37 °C.

PAGE analysis was carried out on 20% polyacrylamide gel and electrophoresis at 280 V for 30 min.
