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Reversible DNA photocircularization on triple helix: effect of vinyl substituent on base stacking

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Abstract

An efficient method for the synthesis of circular oligodeoxyribonucleotides (ODNs) using reversible template-directed photoligation via 5-vinyldeoxycytidine (^{V}C) is described. It is shown that the 5′-end ^{V}C of ODN reversibly reacts by photoirraditation with thymine at its own 3′-end in the presence of a single-stranded template via triple helix formation to produce circular DNA. Thermodynamic analysis indicated that the stability of triple helix is remarkably enhanced by the vinyl substituent of ^{V}C. © 2000 Elsevier Science Ltd. All rights reserved.

Circular DNAs that recognize nucleic acid targets with high affinity and sequence specificity have recently been noted for controlling gene expression owing to their unique binding properties.[1] Circular DNAs are also useful for trapping specific DNA binding proteins, which are used as decoys to sequester disease proteins due to their enzyme resistance.[2] The synthetic method for obtaining circular DNA by enzymatic or chemical ligation has been previously elaborated.[3] Recently we reported a template-directed photoreversible ligation of oligodeoxynucleotides (ODNs) without using toxic chemical reagents, based on the Watson–Crick base pair for the synthesis of circular DNA.[4] We now report herein an alternative synthesis of circular DNA on a triple helix by template-directed photoligation based on the Hoogsteen base pair.

5-Vinyldeoxycytidine (^{V}C)-containing ODNs were synthesized as reported previously.[4b] Incorporation of ^{V}C into oligonucleotides was confirmed by enzymatic digestion and MALDI-TOF MS.[5] When ^{32}P-5′-end-labeled 34 mer d(^{V}CTTTCCACACCTTTCTTTTCTTCACACT-TCTTTT) (ODN **1**) was irradiated at 366 nm in the absence of a template, photocircularization was not observed, but in the presence of template 12 mer ODN **2**, the expected circular ODN **3** was produced in 40% yield as determined by densitometric assay of the PAGE (Fig. 1, lane 3).[6] The same slowly migrating band was detected when ODN **1** was circularized by chemical ligation reported by Kool (lane 5).[3a] From these results, it was confirmed that ODN **3** obtained

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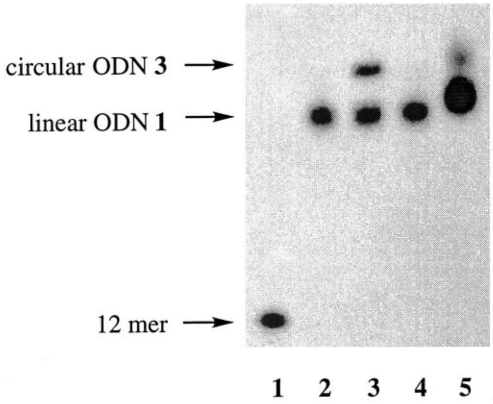
by photoirradiation is a circular ODN linking together at both ends of ODN **1**. A quantitative splitting of the photocircularized ODN **3** was achieved by 302 nm irradiation to regenerate linear ODN **1** (lane 4). This reversible photoligation method would be very useful for synthesizing circular ODNs when enzymatic or chemical methods are undesirable or restricted, such as in a cell (Scheme 1).



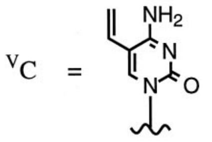
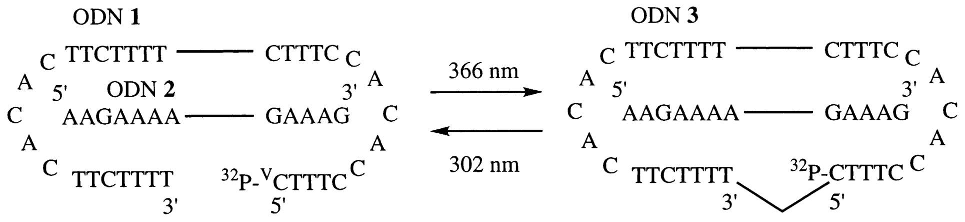


Figure 1. Autoradiogram of a denaturing 20% polyacrylamide gel electrophoresis of the photoreaction of ^{32}P-5′-end-labeled 34 mer ODN **1** (0.5 μM) in the presence of ODN **2** (9 μM). Lane 1, 12 mer control; lane 2, ODN **1** without ODN **2**, irradiation at 366 nm, 3 h; lane 3, ODN **1**+ODN **2**, irradiation at 366 nm, 3 h; lane 4, irradiation of lane 3 at 302 nm, 1 h; lane 5, ODN **1**+ODN **2**, 50 mM BrCn, 100 mM $NiCl_2$, 200 mM imidazole hydrochloride, pH 7.0[3a]



Scheme 1.

To evaluate the effect of ^{V}C at the 5′-terminal of the Hoogsteen strand on the stabilization of triple helix, UV melting studies were carried out using 36 mer hairpin ODN **4** and two 6 mer ODNs **5** and **6a**[7] (Fig. 2a).[8] Their thermal melting behavior exhibited a biphasic helix-coil transition characteristic of triple helix, which is due to a cooperative dissociation of ODN **6a** and ODN **5** from ODN **4**.[9] The melting temperature (T_m) of triple helix is 29.8°C (Fig. 2b). It was also observed that substituting ^{V}C with 5-methyldeoxycytidine (^{Me}C)[10] (ODN **6b**) or C (ODN **6c**) results in a decrease of the thermal stability by 2∼5°C. The thermodynamic parameters obtained from the concentration-dependent thermal denaturing studies are listed in Table 1.[11] The stabilization of the triple helix by incorporating ^{V}C is remarkable and is evidently enthalpic in origin. This stabilizing effect of ^{V}C on triplex formation is due to the enhanced base stacking with the extended π system of ^{V}C.[12] Consistent with this interpretation, molecular modeling studies showed that the vinyl group of ^{V}C stacks onto the 5′-thymine more favorably than onto the 3′-thymine (Fig. 3).[13] This remarkable stacking effect may also be responsible for the selective photoreaction of ^{V}C with 3′-terminal thymine on triple helix.

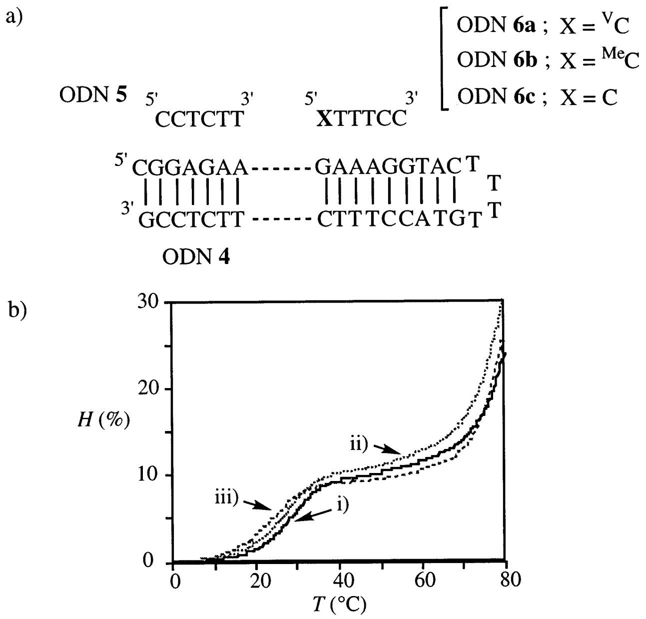


Figure 2. (a) DNA sequences used in this UV melting study. (b) The UV melting curves (H = relative hyperchromicity, $\lambda = 260$ nm) of (i) X = ^{V}C (ODN **6a**; $T_m = 29.8$°C), (ii) X = ^{Me}C (ODN **6b**; $T_m = 28.0$°C) and (iii) X = C (ODN **6c**; $T_m = 24.7$°C) in 1 μM each strand, NaCl 100 mM, $MgCl_2$ 20 mM, 50 mM sodium acetate, pH 5.0

Table 1
Thermodynamic parameters for triplex to duplex transitions of ODN **5** and ODN **6a–c** in the presence of complementary ODN **4**[a]

X	ΔH (kcal/mol)	$\Delta\Delta H$	ΔS (cal/mol K)	$\Delta\Delta S$	ΔG_{298} (cal/mol)	$\Delta\Delta G$
C	51.0±1.3	–	170.3±4.4	–	250.6	–
^{Me}C	51.0±1.3	0	168.9±4.3	−1.4	667.8	+417.2
^{V}C	67.4±1.2	+16.4	222.0±4.1	+51.7	1244	+993.4

[a] All solutions contained 100 mM NaCl, 20 mM $MgCl_2$ and 50 mM sodium acetate (pH 5.0).

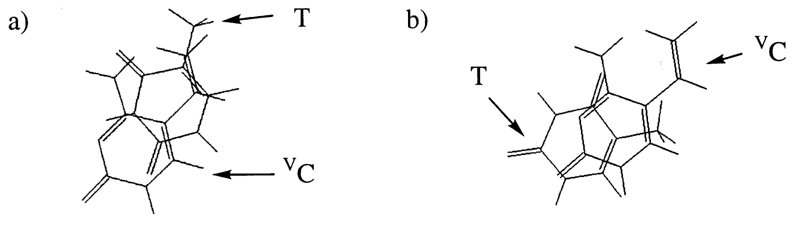


Figure 3. Molecular modeling for base stacking (a) 5′-terminal ^{V}C of ODN **6a** and 3′-terminal thymine of ODN **5**, (b) ^{V}C and thymine of 5′-^{V}CT-3′ sequence of ODN **6a**

In summary, we have demonstrated that reversible photocircularization using ^{V}C is very effective for the synthesis of circular ODNs on triple helix due to the favorable stacking effects, which cannot be accomplished by an enzymatic method.

Acknowledgements

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5. MALDI-TOF MS: calcd for ODN **1** ($C_{330}H_{429}N_{92}O_{216}P_{33}$) ($M-H^-$) 10161.7; found 10160.3.
6. Photoligation was carried out in a 10 μL total volume containing ^{32}P-5′-end-labeled ODN **1** (ca. 2×10^4 cpm, 0.5 μM), ODN **2** (9 μM) in 50 mM sodium acetate buffer at pH 5.0, 100 mM NaCl and 20 mM $MgCl_2$.
7. ESI-TOF MS: calcd for ODN **6a** ($C_{59}H_{77}N_{15}O_{37}P_5$) ($M-H^-$) 1742.330; found 1742.325.
8. The absorbance of the sample (1 μM strand concentration, 50 mM sodium acetate buffer, pH 5.0, 100 mM NaCl and 20 mM $MgCl_2$) was monitored at 260 nm from 2 to 82°C at a heating rate of 1°C per min. For the determination of ΔH and ΔS, melting temperatures of triplexes were measured at seven different concentrations.
9. The biphasic melting curve shown in Fig. 2 is explained by assuming that two 6 mer ODNs cooperatively bind and dissociate onto double-helical ODN **4**. For example, the first melting transition of ODN **6a**/ODN **4** and ODN **5**/ODN **4** exhibited T_m values of 16.8 and 18.2°C, respectively (T_m of the second transition is 78.0°C). Similar results in the melting experiment are also observed with ODN **6b**/ODN **4** ($T_m = 15.5$°C) and ODN **6c**/ODN **4** ($T_m = 10.3$°C). See Ref. 12b,c.
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13. Molecular modeling studies of base-stacking configuration were examined by Amber* force field by Macromodel ver 6.0.