DNA Structures

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Photochemical Synthesis of R-Shaped DNA toward DNA Recombination and Processing In Vitro**

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Ligation of DNA is widely used in DNA repair, replication, and recombination in biomedicine and molecular biology. DNA ligases are enzymes that catalyze formation of the phosphodiester bond between the adjacent 5′-phosphate and 3′-OH groups of two DNA fragments. It is able to join between two single-stranded (ss) DNA fragments annealed on the complementary DNA strand.[1] Although enzymatic ligation methods have advantages, the strategy is limited under certain conditions (such as pH, temperature, and the metal cation) and unique structures, such as branched DNA, cannot be formed. There are methods for nonenzymatic template-directed chemical ligation.[2] These methods use DNA or RNA templates to mediate ligation reactions that generate oligomers of DNA, RNA, or structural analogues of nucleic acids. Modified nucleosides containing various DNA functional groups that react after hybridization by the addition of reagents or by photoirradiation and extended sequence-specific small-molecule synthesis, have been investigated in addition to that of polymerization and single nucleotide polymorphism detection systems.[3,4f] We previously reported a highly efficient and reversible template-directed DNA photoligation of an oligodeoxyribonucleotide (ODN) containing a 5-vinyldeoxyuridine derivative at the 5′ end and an ODN that contains thymine at the 3′ end.[4] In addition, we recently disclosed an efficient template-directed photoligation ODN by using a 5-cyanovinyl-1′-α-2′-deoxyuridine (α^{C}U)-containing ODN at the 3′ end with an ODN that contains thymine at the 5′ end.[5] By using these methods, we succeeded in the synthesis of complex DNA structures, such as branched DNA, end-capped DNA, or padlocked plasmid

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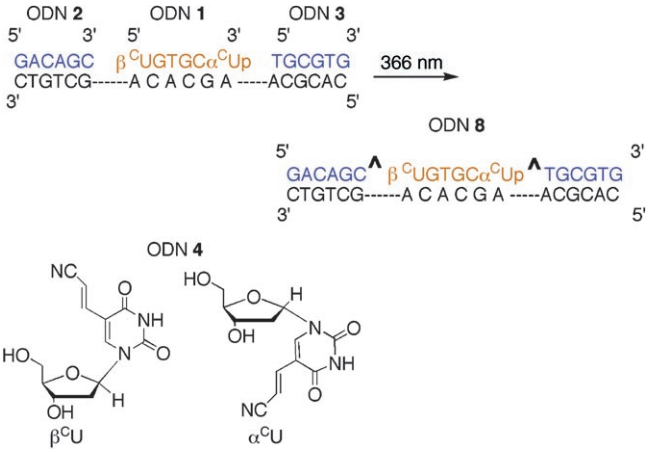
DNA, at desired sites.[6] Although template-directed chemical ligation and photoligation connect modified DNA to natural DNA, they are not able to connect two natural DNA molecules. Therefore, these methods are subject to limitation with respect to the applications such as biotechnological techniques. If two natural DNA molecules can be connected, the photoligation method would become a useful strategy for biotechnological application in combination with the usual gene manipulation. Thus, we developed a new ligation method that cross-links two natural DNA molecules. Herein, we report template-directed photoligation by using ODNs that contain α^{C}U and β^{C}U at both terminal ends. By using this method, two unmodified ODNs as natural DNA were photochemically ligated through a modified ODN in the presence of template ODN. Furthermore, we developed a method that can directly process DNA in a method similar to RNA processing. We applied the method to the synthesis of R-Shaped DNA as a unique structure to resemble lariat DNA.[7]

The synthesis of the photoreactive nucleosides and the corresponding phosphoramidite building block of α^{C}U and 5-cyanovinyl-1′-β-2′-deoxyuridin (β^{C}U) as well as the synthesis of the corresponding ODN followed standard routes in DNA chemistry. α^{C}U was synthesized from Hoffer's chlorosugar; the method was reported previously,[5] and the synthesis of β^{C}U is shown in the Supporting Information. The modified ODNs containing α^{C}U and β^{C}U (5′-d(β^{C}UGTGCα^{C}Up)-3′ (ODN **1**) and 5′-d(β^{C}UGCATGTGCα^{C}Up)-3′ (ODN **5**)) were synthesized by using a 3′-phosphate controlled pore glass (CPG) solid-phase support.[8] After purification with reversed-phase HPLC, ODN **1** was characterized by MALDI-TOF MS (*m*/*z* calcd for $[M-H]^-$: 1950.2828; found: 1950.5432). The modified ODN **5** was characterized by MALDI-TOF MS (*m*/*z* calcd for $[M-H]^-$: 3189.0578; found: 3189.0632). The ODNs used in this study are summarized in Table 1.

Table 1: ODNs used in this study.

ODN	Sequences
ODN **1**	5′-d(β ^{C}UGTGCα^{C}Up)-3′
ODN **2**	5′-d(GACAGC)-3′
ODN **3**	5′-d(TGCGTG)-3′
ODN **4**	5′-d(CACGCAAGCACAGCTGTC)-3′
ODN **5**	5′-d(β?^{C}UGCATGTGCα^{C}Up)-3′
ODN **6**	5′-d(GAGACGTGAT)d(A)$_{20}$(TGGCAGTACG)-3′
ODN **7**	5′-d(CGTACTGCCAAGCACATGCAATCACGTCTC)-3′

We determined the feasibility of the template-directed photoligation through ODNs that contain α^{C}U and β^{C}U at the 3′ and 5′ ends, respectively (Scheme 1). 5′-d(GACAGC)-3′ (ODN **2**), 5′-d(TGCGTG)-3′ (ODN **3**), and ODN **1** were irradiated at 366 nm for 1 h at 0 °C in the presence of template ODN **4**. Capillary-gel-electrophoresis (CGE) analysis of this mixture indicated a clean and efficient formation of ligated ODN **8** and the concomitant disappearance of ODN **1**, ODN **2**, and ODN **3** (Figure 1). We observed the appearance of the peak of ligated product ODN **8** in 95 % yield as determined by CGE analysis. The yield was calculated based on the average



Scheme 1. Template-directed photoligation of ODNs through α^{C}U and β^{C}U. The connections made by photoirradiation in ODN **8** are indicated (**Λ**).

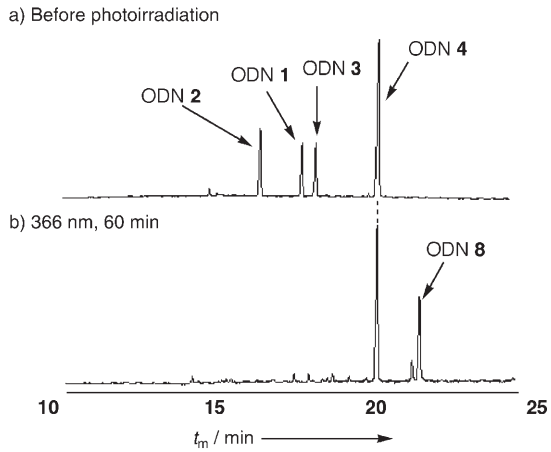


Figure 1. CGE of ODN **1**, ODN **2**, and ODN **3** irradiated at 366 nm in the presence of template ODN **4**. a) Before photoirradiation, and b) after irradiation at 366 nm for 60 min, 95 % yield. t_m = migration time.

of ODN **2** and ODN **3**. MALDI-TOF MS indicated that ODN **8** was a ligated product of ODN **1**, ODN **2**, and ODN **3** (*m*/*z* calcd for $[M-H]^-$: 5575.73; found: 5575.70). The molecular weight of ODN **8** was equal to the sum of the molecular weights of ODN **1**, ODN **2**, and ODN **3**. Enzymatic digestion of isolated ODN **8** with P1 nuclease, snake venom phosphodiesterase, and alkaline phosphatase showed the formation of deoxycytidine (dC), deoxyguanosine (dG), thymidine (T), and deoxyadenosine (dA) in a ratio of 3:7:2:2 together with new products (see Supporting Information). Spectroscopic data, including MALDI-TOF MS, indicated that these new products were the α^{C}U-T (*m*/*z* calcd for $[M+H]^+$: 522.1836; found: 522.1999) and dC-β^{C}U (*m*/*z* calcd for $[M+H]^+$: 507.1839; found: 507.2011) photoadducts. The structure of α^{C}U-T and dC-β^{C}U was expected to be a [2+2] adduct on the basis of previous reports.[4c,5] Although two unmodified 6-mer ODNs were ligated to modified ODN **1** in the presence of template ODN, this result suggests that the new method could

apply to the ligation of two DNA or RNA fragments, such as DNA recombination in vitro.[9]

To expand the new system, we performed template-directed photoligation at two positions in the middle of the DNA structures, and synthesized R-shaped DNA as a novel structure (Scheme 2). ODN **5** and ODN **6** were irradiated at 366 nm for 2 h at 0 °C in the presence of template ODN **7**. Figure 2b shows a CGE analysis of a photoirradiated mixture with clean and efficient formation of the expected ligated 50-mer R-shaped ODN **9** and the complete disappearance of ODN **5** and ODN **6**. We observed the appearance of the peak of the ligated product ODN **9** in 90% yield as determined by CGE analysis. The yield was calculated based on the average yield of ODN **5** and ODN **6**. As shown in Figure 3, the generation of the product with one end connected is confirmed by priority for the first 20 minutes (as the slope of residual ratio for one-end-connected product (red line) in the first 20 min in Figure 3 is steeper than the slope of yield for R-shaped DNA (orange line)). The increase of ODN **9** was more gradual than the product with one end connected. These results suggest that photoligation progressed stepwise from the product with one side connected to ODN **9**. We considered that the second reaction was promoted by an increase in the stability of the duplex that is formed by the product with one end connected. The isolated new product was characterized by MALDI-TOF MS (m/z calcd for $[M+H]^+$: 15676.36; found: 15676.76) and enzymatic digestion (see Supporting Information). As a result, we confirmed that ODN **9** is R-shaped DNA. The method of R-shaped DNA synthesis may offer novel DNA processing in vitro that is modelled on pre-mRNA splicing.

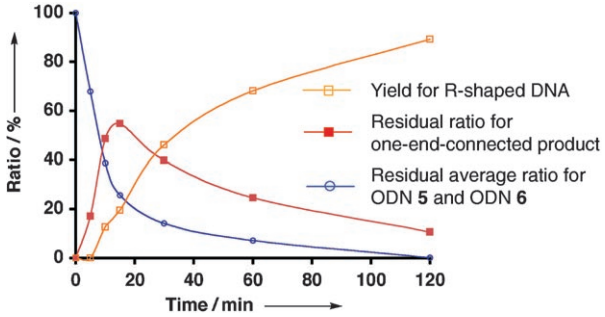
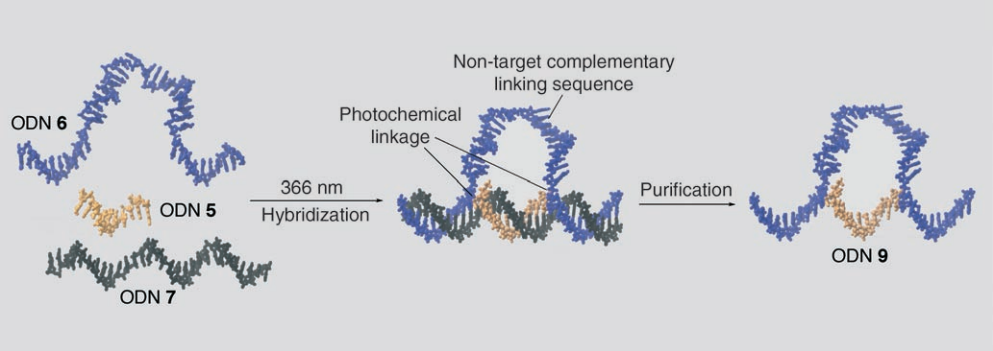


Figure 3. Yield for R-shaped DNA and residual ratio for ODN **5**, ODN **6**, and the one-end-connected product as determined by CGE.



Scheme 2. Template-directed photoligation synthesis of R-shaped DNA through α^CU and β^CU

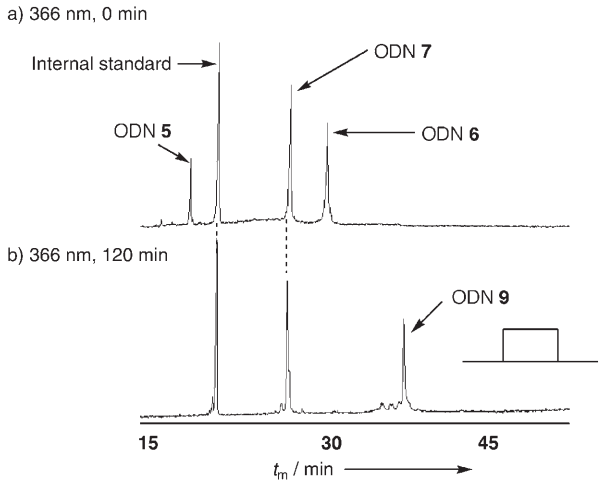


Figure 2. CGE of ODN **5** and ODN **6** irradiated at 366 nm in the presence of template ODN **7**. a) Before photoirradiation, and b) after irradiation at 366 nm for 120 min, 90% yield.

In conclusion, we demonstrated a novel strategy for connecting DNA strands by using ODNs that contain α^CU and β^CU at the 3′ and 5′ ends, respectively. Two DNA fragments were ligated to the modified ODN that contains α^CU and β^CU in the presence of template ODN. These methods can be used for recombination that mutually connects two or more genes of different kinds. We have also shown the synthesis of a novel unique DNA structure by R-shaped ODN. These methods may be used for the in vitro DNA processing of any DNA, such as genome DNA and plasmid DNA. Additionally, R-shaped DNA may construct the periodically controlled rod- and sheet-type nanoarrays[10] and DNA nanodevices[11] as part of the nanostructures. These unique structures are a powerful tool for easy and accurate DNA handling, leading to the development of DNA nanoarchitecture as represented by various crossover DNA motifs.

Experimental Section

Photoligation of ODNs as monitored by CGE: The reaction mixture (total volume = 100 μL) containing ODN **1**, ODN **2**, and ODN **3** (each with a strand concentration of 30 μM) in the presence of template ODN **4** (strand concentration of 33 μM,) 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. After irradiation, the progress of photoreaction was monitored by CGE on a BECKMAN COULTER, P/ACE™ MDQ Capillary Electrophoresis system. The denaturing gel (eCAP™ ssDNA 100-R

Kit) purchased from BECKMAN COULTER containing Tris-Borate buffer solution and urea was prepared according to the instruction manual. The enzymatic digestion was carried out with alkaline phosphatase, phosphodiesterase in NaCl (100 mM), Tris-HCl (100 mM; pH 8.9), 50% glycerol, $MgCl_2$ (15 mM), and P1 nuclease at 37°C for 4 h.

Spectroscopic measurements: The absorbance of the hybrid duplexes was monitored at 260 nm from 10 to 80°C with a heating rate of 1.0°C min^{-1} by using a BECKMAN COULTER DU 800 UV/Vis spectrophotometer.

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