Rapid Photopolymerization of Oligodeoxynucleotides by 3-Cyanovinylcarbazole mediated DNA Photocrosslinking

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We proposed rapid photopolymerization of oligodeoxynucleotides (ODNs) by 3-cyanovinylcarbazole (CNVK), which photoradiates the crosslinking between DNA strands at 366 nm. This photopolymerization of ODNs using CNVK was completed by the photoirradiation for within two seconds, the reaction rate of which is faster than any other method such as enzymatic and chemical ligation. Moreover, the photopolymerized DNA structure was degraded to the monomer ODN by photoirradiation at 312 nm. The photopolymerized DNA structure has B-form the same as the native helical duplex structure and its structure attained thermal resistance when maintained at a temperature of 60 °C. Sequence specific photopolymerization incorporating micro RNA was also successful.

Keyword: 3-cyanovinylcarbazole, ultrafast photocrosslink, DNA structure, micro RNA, photoreversibility

1. Introduction

A photopolymer is a molecule that changes its chemical properties and molecular weight by photoirradiation. Photopolymerization is a widely used technology in applications for photoresist, dental treatment, and 3D-image print materials [1-3]. The monomer residues such as polyvinyl cinnamate and acrylate epoxides are polymerized by photoirradiation [4,5]. Researches on investing new materials for application with high sensitivity and size regulation have received much attention in recent years.

DNA is one of the most important biopolymers in living cells. This biopolymer is consists four deoxynucleotides such as adenine(A), thymine(T), guanine(G), and cytosine(C), and forms a helical duplex structure in cell. Two DNA polymers with complementary base sequences can be paired following the strict Watson-Crick rule, A-T and G-C, resulting in the formation of the DNA double helix. The structure and conformation of DNA can be rationally programmed by the sequence design [6,7]. It was possible to regulate the structure on the nano-scale as shown in the DX tile and DNA origami reported by Seeman and Rothemund [8-12]. However, these structures are self-assembled by the hydrogen bond without the covalent bond.

In contrast, a polymerization method from short DNA strand using enzyme and thymine dimer has been reported. A long DNA strand was created from a short oligodeoxynucleotide (ODN) strand or monomer residues by the ligase and polymerase [13-16]. In 1982, Lewis and Hanawalt reported the ligation of ODNs by pyrimidine dimers using photoirradiation [17,18]. A nucleic acid polymer based on the photodimerization of pyrimidine base units was also reported toward photoresists [19]. When the research was first strand, this photopolymerization based on thymidine dimer required long photoirradiation, and the number
of polymerization was negligible. Later, some photoresponsive artificial nucleotides based on the thymidine dimer were reported to be photopolymerized from ODNs with a shorter photoirradiation time [20,21].

We have already reported on the photoresponse in artificial nucleotide 3-cyanovinylcarbazole (CNVK), which can photocrosslink to a complementary DNA strand via [2+2] photocycloaddition between CNVK and pyrimidine base in a complementary strand with 1 second of 366 nm irradiation [22,23]. We demonstrated the photopolymerization of ODNs using CNVK irradiation at 366 nm and photodegradation of photopolymerized DNA irradiation at 312 nm. We also demonstrated the feasibility of photopolymerized DNA-RNA hetero-duplex incorporating micro RNA (miRNA) in a sequence specific manner.

2. Experimental

2.1. General

Mass spectra were recorded on Voyager-DE PRO-SF, Applied Biosystems. Irradiation was performed by UV-LED(366 nm, 1,600 mW/cm², ZUV, OMRON, Japan) and transilluminator(312 nm, Funakoshi). CD spectra were measured on a JASCO J-720 spectropolarimeter. HPLC was performed on an InterSustain™ C18 column(GL Science, 5 µm, 10 × 150 mm) or an InterSustain™ C18 column(GL Science, 5 µm, 4.6 × 150 mm) with a JASCO PU-980, HG-980-31, DG-980-50 system equipped with a JASCO UV-970 detector at 260 nm. Reagent for the DNA synthesizer such as A, G, C, T-β-cyanoethyl phosphoramidite, and CPG support were purchased from Glen Research.

2.2. DNA sequences, synthesis and purification

Oligonucleotides were prepared by β-(cyanoethyl) phosphoramidite method on controlled pore glass supports (1 µmol) using DNA synthesizer(AppliedBio). Strands containing CNVKs were synthesized using the cyanoethylphosphoramidite of CNVK that was synthesized according to the previous reported method[20]. After automated synthesis, the oligomer was detached from the support by soaking in concentrated. aqueous ammonia for 1 h at room temperature. Deprotection was conducted by heating the concentrated aqueous for 4 h at 65°C concentrated aqueous ammonia was then removing it by speedvac, and the crude oligomer was purified by reverse phase HPLC and lyophilized.

2.3. Photoirradiation condition of photopolymerization and photodegradation

Each ODNs including CNVK was dissolved to 12.5 µM in annealing buffer (10 mM MgCl₂, 20 mM Tris-acetate (pH 7.0), 1 mM, EDTA). After mixing, the solutions were photoirradiated at 366 nm UV-rays on ice using a UV-LED illuminator. The 312 nm photoirradiation was performed under the heating at 50°C using a transilluminator.

2.4. Denaturing polyacrylamide gel electrophoresis

For analysis of the sizes of photopolymerized DNA structure by gel electrophoresis, sample solutions were diluted by 8M Urea in formamide and heated at 90°C for 5 min. Loading samples were prepared with an appropriate amount of 6×loading buffer [36% (v/v) glycerol, 30 mM EDTA and 0.05% (w/v) each of bromophenol blue and xylene cyanol] and loaded onto gels prepared with 10% polyacrylamide (29:1, polyacrylamide: bisacrylamide) containing 8 M Urea and running buffer (1×TBE). 25 bp DNA Ladder Maker (Promega) was used. The gels were run at 150 V on a gel electrophoresis apparatus (Bio-RAD). After electrophoresis, the gels were stained with SYBRgold (Molecular Probe) and imaged on an Imaging System LAS-3000 (FUJIFILM Inc.).

2.5. Agarose gel electrophoresis

3 µl of each same DNA was electrophoresed on a 3 % agarose gel at 100 V in TAE buffer at constant current for 30 min with Mupid-2plus(Mupid). 100 bp DNA Ladder Maker (CosmoBio) was used. After electrophoresis, the gels were stained with SYBRgold (Molecular Probe) and imaged on LAS-3000(FUJIFILM Inc.)

2.6. Circular dichroism spectrum

Circular dichroism spectrum were determined in 0.1 cm path length cells using a J-720 spectrophotometer (JASCO), equipped with a thermoelectric temperature controller.

2.7. Annealing and photoirradiation condition of photopolymerization incorporating miRNA

12.5 µM Probe ODN and 12.5 µM miRNA was dissolved in annealing buffer and
photoirradiated at 25°C or 37°C for 120 s.

3. Result and discussion

At first, based on the sequence that was constructed of 1D DNA with two strands, the DNA strand ODN 1 (5'-GACTGTCCGATGTA\textsuperscript{CNVK}TGCGATGTA\textsuperscript{CNVK}KTCGG-3') and ODN 2 (5'-GCCGA\textsuperscript{CNVK}KAGT CCGCGATTCGTGTA\textsuperscript{CNVK}KGCACATACAT-3') including \textsuperscript{CNVK}K were designed to create a photopolymerized 1D DNA structure (Scheme 1). The \textsuperscript{CNVK}K photocrosslinked to thymidine in a complementary strand, and the strand ODN 1 were separated into two domains, \(a\) and \(b\) whose sequences are a complementary sequence of \(\bar{a}\) and \(\bar{b}\) domains in strand ODN 2. In this strategy, each strand was hybridized with the other strand and this propagates the next chain reaction of hybridization and photocrosslinking events between alternating ODN 1 and 2 to form a nicked double-helix.

To confirm the photopolymerization, two ODNs including \textsuperscript{CNVK}K were photoirradiated at 366 nm, and analyzed by denaturing PAGE and agarose gel electrophoresis (Fig. 1A,B). As shown in Fig. 1A, the only monomer band was observed in the non-photoirradiated sample (Lane 2) and bands having low mobility compared with the monomer band appeared and the mobility of these bands decreased depending on the photoirradiation time, suggesting that the start monomer ODN 1 and 2 were photopolymerized via a photocrosslinking between \textsuperscript{CNVK}K and thymidine. To confirm the size of this photopolymer, it was analyzed by 3% agarose gel electrophoresis (Fig.1B). As shown in Fig. 1B, the photopolymerization process was completed by irradiation for 2 seconds, and the size of the photopolymerized DNA structure was 500±300 bp. The rate of photopolymerization using \textsuperscript{CNVK}K was 250 bp per second, which was approximately 125-fold faster than the extension rate of the enzymatic method.

We previously reported that this photocrosslinking reaction of \textsuperscript{CNVK}K had reversibility by irradiation at 312 nm. So, to confirm the photoreversibility, the photopolymerized DNA was irradiated at 312 nm at 50 (Scheme 2) and analyzed by

![Scheme 2. Photodegradation of photopolymerized DNA to monomer ODNs by photoirradiation at 312 nm.](image)
Figure 2. Degradation of photopolymerized DNA by photoirradiation at 312 nm. Lane M is 25 bp DNA Ladder Maker. Lane 1 to Lane 12 are samples irradiated with 312 nm for 0, 1, 3, 5, 10, 30, 60, 120, 300, 600, 900, and 1800 seconds at 5°C respectively.

...denaturing PAGE (Fig. 2). As shown in Fig. 2, the band of photopolymerized DNA disappeared depending on the photoirradiation time and the only monomer band was observed in Lane 12 after photoirradiation at 1800 s, suggesting that this photopolymerized DNA was reversibly degraded to monomer ODN by photosplitting.

Next, the conformation of this photopolymerized DNA was examined by CD spectrum measurement. The CD spectrums of photopolymerized DNA are shown in Fig. 3A, the measurement at 10°C is shown as a solid line. The CD spectrums had a negative cotton effect at 255 nm and positive effect at 285 nm, suggesting that this photopolymerized DNA had B-form the same as the native DNA duplex. Moreover, this photopolymerized DNA was measured by CD spectrum at 20°C, 30°C, 40°C, 50°C, and 60°C to examine the thermal stability. The cotton effect of photopolymerized DNA at 255 nm was not reduced compared with the cotton effect of starting ODN 1 and ODN 2, so that its B-form was maintained by heating at 60°C. These results suggest the conformation of photopolymerized DNA was stable B-form DNA caused from \([2+2]\) cycloaddition between CNVK and thymidine created by the photocrosslinking reaction.

Next, we demonstrated the feasibility of sequence specific photopolymerization of DNA or RNA for rapid sensing of nucleic acids (Scheme 2). In this experiment, we used five miRNA that belong to the miRNA 200 family as target strands. The probe ODNs were designed to polymerize only in the presence of target miRNA by photoirradiation (Table 1). For the positive detection of the SNPs, a probe ODN complementary to each miRNA sequence was designed and each probe ODN has two CNVKs that photocrosslink to two target miRNAs, respectively. To examine the photopolymerization incorporating the miRNA in a sequence specific manner, we demonstrated the photopolymerization in all combinations (5 probes × 5 miRNAs). The samples were photoirradiated at 366 nm for 120 seconds and analyzed by denaturing PAGE. The band intensity of the denaturing PAGE result was normalized by the sum of the band intensity. The
Table 1. The sequence of target miRNAs and probe ODNs.

<table>
<thead>
<tr>
<th>Entry</th>
<th>The sequence of strand</th>
</tr>
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<tbody>
<tr>
<td>200a</td>
<td>UAACACUGUCUGGUAACAGAUAGU</td>
</tr>
<tr>
<td>200b</td>
<td>UAUAUACUGCCUGGUAACAGAUAGU</td>
</tr>
<tr>
<td>200c</td>
<td>UAUAUACUCGCGGUAACAGAUAGU</td>
</tr>
<tr>
<td>141</td>
<td>UAACACUGUCUGGUAACAGAUAGU</td>
</tr>
<tr>
<td>429</td>
<td>UAUAUACUGUCUGGUAACAGAUAGU</td>
</tr>
</tbody>
</table>

normalized band intensity of each number of polymerization is shown in Fig. 4. In the case of probe ODN 200a, the photopolymerization reaction was advanced in the presence of miRNA 200a. However, only monomer and dimer were observed in the presence of other miRNAs. And, all probes were photopolymerized incorporating miRNA only in the presence of target miRNA, respectively. The result clearly shows that the sequence specific photopolymerization incorporating miRNA was successful toward miRNA sensing.

4. Conclusion

In this paper, we propose ultrafast photopolymerization of ODNs using CNVK as the photoresponse artificial nucleoside. These results show that a stable DNA photopolymer was successfully created from short ODNs rapidly by 366 nm irradiation, and its photopolymer was degraded to start short ODNs by 312 nm irradiation, respectively. And, this photopolymerization can create a DNA-RNA hetero polymer incorporating miRNA in a sequence specific manner. Development of this technology might provide us with new interesting material for DNA nanotechnology and DNA engineering. Moreover, this rapid sequence specific photopolymerization incorporating miRNA will also be used for miRNA sensing, and this technology might provide us with diagnostic tool and nucleic acid medicine for the treatment of diseases.

![Figure 4. The number of polymerization of photopolymerization DNA in all combination (5 probes and 5 miRNAs). The abscissas axis shows the number of polymerization and ordinat axis shows the normalized band intensity. * is photoirradiated at 37°C, non-sign is photoirradiated at 25°C.](image)
Acknowledgments

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Reference