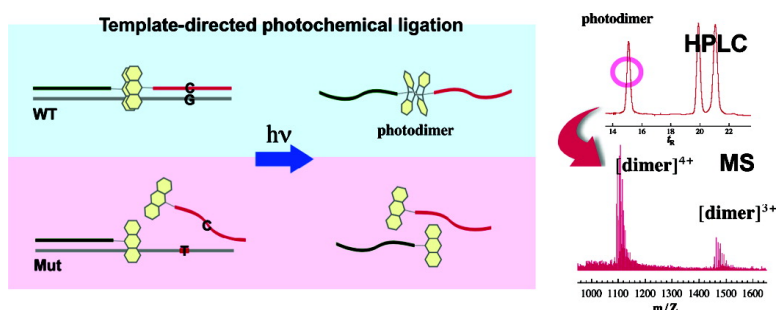## Communication

Photochemical Ligation of DNA Conjugates through Anthracene Cyclodimer Formation and Its Fidelity to the Template Sequences

Toshihiro Ihara, Tomohiro Fujii, Motoko Mukae, Yusuke Kitamura, and Akinori Jyo

More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 3 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Photochemical Ligation of DNA Conjugates through Anthracene Cyclodimer Formation and Its Fidelity to the Template Sequences

Toshihiro Ihara,* Tomohiro Fujii, Motoko Mukae, Yusuke Kitamura, and Akinori Jyo

Department of Applied Chemistry and Biochemistry, Faculty of Engineering, Kumamoto University, 2-39-1 Kurokami, Kumamoto 860-8555, Japan

Received April 17, 2004; E-mail: toshi@chem.kumamoto-u.ac.jp

Chemical ligation, nonenzymatic joining of DNA ends by covalent bonds, has attracted a great deal of interest because of its potentials in biomedical and synthetic applications.[1] The techniques involved should also be applicable for producing DNA-based unique nanostructures.[2] Although various methods for template-directed chemical ligation have been reported, there have been only a limited number of reports concerning ligations based on photochemical reactions.[3] Photoligation has several advantages including the lack of a need for additives, low cost, and ease of reaction control by wavelength, light strength, and irradiation time.

Here, we attached an anthracene group as a handle to one end of each of a number of oligodeoxyribonucleotides (ODNs) to make anthracene−ODN conjugates. Anthracene readily forms photo-adducts, anthracene dimers, and this photodimerization reaction has been characterized in detail.[4] The reactivities of many anthracene derivatives under various conditions have been studied extensively. Although the yields of anthracene dimer formation in diluted solutions are generally low, the reactions proceed efficiently under particular conditions, such as in the cavity of cyclodextrin because of the concentration effect in a microenvironment.[5] DNA should function as a good scaffold to provide such a microenvironment if the system is designed carefully for close proximity and spatial alignment of the reaction partners.

The structures and the sequences of the four ODN conjugates and the templates used in the present study are shown below.
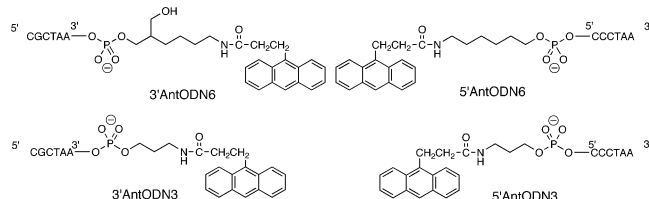
WT: TTAGGGTTAGCG
Gap1: TTAGGG**T**TTAGCG   **Mut1:** TTAGGG**A**TAGCG
Gap2: TTAGGG**TTT**TAGCG   **Mut2:** TTAGGGT**A**AGCG
Gap3: TTAGGG**TTTT**TAGCG   **Mut4:** TTAGGGTTA**T**CG

5′AntODN6 and **3′AntODN6** are ODN conjugates bearing anthracene units on their 5′- and 3′-ends, respectively, through a hexamethylene linker chain. **5′AntODN3** and **3′AntODN3** are the corresponding conjugates with a trimethylene linker. The sequences of the conjugates, **5′AntODN***n* and **3′AntODN***n* (*n* = **3** or **6**), were designed to bind adjacent sequences of the template, **WT**, with the anthracene units directed such that they stacked with each other.

UV melting experiments were carried out for the ODN conjugates and unmodified ODNs with the same sequences (Figure 1a). The apparent melting temperature of the tandem duplex of the conjugates (25.5 °C) was higher than that of unmodified ODNs (18.5 °C) by ca. 7 °C. The anthracene units markedly contributed to the thermal stability of the tandem duplexes probably due to their mutual $\pi-\pi$
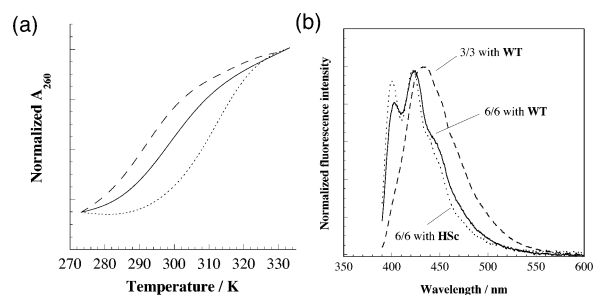

Figure 1. Preliminary studies of ODN interactions. (a) UV melting curves recorded at 260 nm for the duplexes with **WT**. Solutions containing each component at 1 μM were made with 1 mM phosphate buffer (pH 6.5) and 1 M NaCl, and subjected to melting experiments. The solutions were heated at a rate of 0.5 deg min^{-1} after annealing. Broken curve, tandem duplex of unmodified 6-mers with **WT**; solid curve, 6/6 tandem duplex with **WT**; dotted curve, duplex of the photodimer and **WT**. (b) Emission spectra of the solutions of 0.5 μM tandem duplexes with **WT**, measured at 0 °C in the same buffer as was used in the melting experiments. Solid curve, 6/6 tandem duplex with **WT**; broken curve, 3/3 tandem duplex with **WT**; doted curve, sum spectrum of each conjugate duplex with corresponding **HSc**s. λ_{ex} = 380 nm.

stacking interaction or favorable interactions with the duplex structure. Emission spectra of the tandem duplexes were measured at 0 and 40 °C. The spectra observed at both temperatures were different from each other. While the spectra at 40 °C were almost the same with the sum of the monomer emissions, those at 0 °C were shifted to longer wavelengths. The conjugates place two anthracene chromophores in proximity and give rise to excimer emission. The spectra were measured for all four possible combinations. The extent of the spectral shift was decreased in the order 3/3 ≅ 6/3 > 6/6 ≅ 3/6 (*n/n* represents the combination of the conjugates, **5′AntODN***n*/**3′AntODN***n*) (Figure 1b).

Figure 2a shows reversed phase HPLC chromatograms of solutions of the tandem duplex, 6/6, before and after irradiation. A new peak that appeared at t_R = 13 min after irradiation was identified as the conjugate photodimer by MALDI- and ESI-TOF/MS spectrometry (calcd for [6/6 dimer]$^{4+}$: 1091.76, found: 1091.73). Photoirradiation was essential for this reaction. In addition, the dimer was not observed even in trace quantities without the template, **WT**. Time courses of photodimerization for the 6/6 and 3/3 tandem duplexes are indicated in Figure 2b. It was obvious that 6/6 dimer was generated more rapidly than the 3/3 dimer. The initial rate constants of the first-order kinetics were 1.13×10^{-4} ($t_{1/2}$ = 102 min) and 2.73×10^{-5} s^{-1} ($t_{1/2}$ = 423 min) for 6/6 and 3/3 tandem complexes, respectively. Relative yields of photodimer after 30 min of irradiation were 3/6 (1.46) > 6/6 (1.00) > 6/3 (0.33) > 3/3 (0.24).[6] This reactivity sequence was the opposite to the strength of excimer emission. Anthracenes photodimerize by way of the singlet excimer. That is, excimer formation is a prerequisite for the photodimerization of anthracenes. However, detection of
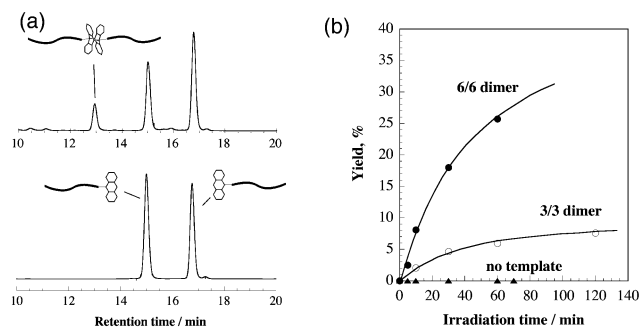
Figure 2. Photoligation studies. Solutions containing each component at 30 μM were made with 1 mM phosphate buffer (pH 6.5) and 1 M NaCl and irradiated in an Ar atmosphere (0 °C) at 366 nm using a high-pressure mercury lamp equipped with appropriate optical glass filters. (a) HPLC chromatograms (detected by the absorbance at 260 nm) of the 6/6 reaction mixtures before (bottom) and after 30 min (top) of irradiation. Column, Shodex ODP-50 6D; solution A, 0.1 M ammonium acetate (pH 9); solution B, acetonitrile; linear gradient, 10−50% in 30 min; flow rate, 1 mL/min. (b) Time courses of the photodimerization yields. Closed circles, 6/6 mixture with **WT**; open circles, 3/3 mixture with **WT**; triangles, 6/6 mixture without template. The yields were determined by the peak area on the HPLC chromatograms.

Table 1. Ligation Efficiencies between **5′AntODN6** and **3′AntODN6** (6/6) in the Presence of Various Templates[a]

template	rel. efficiency	template	rel. efficiency
WT	1.00	**HSc**	0.00
Gap1	1.10	**Mut1**	0.35
Gap2	0.16	**Mut2**	0.19
Gap3	0.06	**Mut4**	0.00

[a] Yield relative to that of the tandem duplex with **WT** after 30 min of photoirradiation at 0 °C. The conjugates were added to the reaction mixtures as the duplexes with complementary 6-mers (competitors).

the excimer by its fluorescence should not be taken to indicate that dimerization will necessarily occur, because excimer emission itself occurs via another pathway from the mutual intermediate, singlet excimer.[4]

The isolated photodimer was subjected to melting analysis. The melting behavior is shown in Figure 1a. The apparent melting temperature of the conjugate dimer was 39.1 °C, which was 13.6 °C higher than that of the tandem duplex. This substantial stabilization was due to the difference in length; two 6-mer conjugates were cross-linked with each other to form a 12-mer ODN conjugate.[7]

The efficiencies of photoligation were examined for 6/6 dimers with several templates. The efficiencies relative to that with the template **WT** are summarized in Table 1. No photodimers were detected in the sample with the template carrying half-scrambled sequence, **HSc**. This indicated that the photoligation observed in this study required that both conjugates hybridize to adjacent sites. The effect of the length of the intervening gap between the two sites was also examined. While one nucleotide gap (**Gap1**) was allowed for efficient ligation, the reactivity was significantly reduced for ligation with the templates carrying longer gaps (**Gap2** and **Gap3**). The optimum length of the gap was much shorter than that expected from the length of the linker chains of the two ODN

conjugates, **5′AntODN6** and **3′AntODN6**; the length of the linker chain of the 6/6 photodimer corresponds to 6−7 nucleotides. Appropriate slack (freedom) in the chains seems to be required for efficient dimerization by the entropic demand in terms of statics. In addition, the dynamics of several processes, mainly emission and dimerization, from the common intermediate should also be taken into account to explain the optimum distance between the conjugates for efficient ligation.

Interestingly, the photodimerization efficiency was also affected by one-base displacement in a position-dependent manner. The efficiency decreased in the order of **WT** > **Mut1** > **Mut2** > **Mut4**. That is, mismatch in the central positions (**Mut4**) reduced the efficiency to a greater extent than those at the ends (**Mut1** and **Mut2**). The order of efficiencies coincided with that of the thermal stabilities of the duplexes.[8]

In conclusion, we have demonstrated that two anthracene−ODN conjugates could be ligated through anthracene photodimer formation. The reactivity showed high fidelity to the sequence of the template. Especially, the difference in efficiency between **WT** and **Mut4** was sufficiently large to be applied for SNP detection if the method is combined with MS spectroscopy.

Supporting Information Available: Details regarding synthesis of the anthracene−ODN conjugates, mass spectrum of the dimer. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Xu, Y.; Kool, E. T. *J. Am. Chem. Soc.* **2000**, *122*, 9040 and references therein. (b) Xu, Y.; Karalker, N. B.; Kool, E. T. *Nat. Biotechnol.* **2001**, *19*, 148. (c) Czlapinski, J. L.; Sheppard, T. L. *J. Am. Chem. Soc.* **2001**, *123*, 8618. (d) Herrlein, M. K.; Nelson, J. S.; Letsinger, R. L. *J. Am. Chem. Soc.* **1995**, *117*, 10151. (e) Gartner, Z. J.; Grubina, R.; Calderone, C. T.; Liu, D. R. *Angew. Chem., Int. Ed.* **2003**, *42*, 1370.

(2) (a) Mirkin, C. A. *Inorg. Chem.* **2000**, *39*, 2258. (b) Niemeyer, C. M. *Curr. Opin. Chem. Biol.* **2000**, *4*, 609. (c) Sha, R.; Liu, F.; Millar, D. P.; Seeman, N. C. *Chem. Biol.* **2000**, *7*, 743. (d) Ihara, T.; Chikaura, Y.; Tanaka, S.; Jyo, A. *Chem. Commun.* **2002**, 2152. (e) Ihara, T.; Takeda, Y.; Jyo, A. *J. Am. Chem. Soc.* **2001**, *123*, 1772. (f) Soto, C. M.; Srinivasan, A.; Ranta, B. R. *J. Am. Chem. Soc.* **2002**, *124*, 8508. (g) Patolsky, F.; Weizmann, Y.; Lioubashevski, O.; Willner, I. *Angew. Chem., Int. Ed.* **2002**, *41*, 2323.

(3) (a) Liu, J.; Taylor, J.-S. *Nucleic Acids Res.* **1998**, *26*, 3300. (b) Fujimoto, K.; Matsuda, S.; Takahashi, N.; Saito, I. *J. Am. Chem. Soc.* **2000**, *122*, 5646.

(4) For example: (a) Gilbert, A.; Baggott, J. In *Essentials of Molecular Photochemistry*; CRC Press: Boca Raton, 1991; Chapter 8. (b) Bouas-Laurent, H.; Desvergne, J.-P. In *Photochromism, Molecules and Systems*; Dürr, H., Bouas-Laurent, H., Eds.; Elsevier: Amsterdam, 1990; Chapter 14.

(5) Nakamura, A.; Inoue, Y. *J. Am. Chem. Soc.* **2003**, *125*, 966.

(6) Unknown byproducts were observed in chromatograms by light irradiation over 2 h. Singlet oxygen would be responsible, at least in part, because degassing was effective to suppress this side reaction.

(7) The melting temperature of plain 12-mer ODN with the same sequence was 52.3 °C. Although both 12-mer ODNs, the conjugate photodimer and the native 12-mer, had the same sequence, the long connector including the anthracene dimer seems not to be favorable for hybridization, probably due to steric hindrance.

(8) (a) Ketomäki, K.; Hakala, H.; Lönnberg, H. *Bioconjugate Chem.* **2002**, *13*, 542. (b) Peyret, N.; Seneviratne, P. A.; Allawi, H. T.; SantaLucia, J., Jr. *Biochemistry* **1999**, *38*, 3468 and references therein.

JA047784R