Supramolecular Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713649759

Photodimerisation of anthracenes using a DNA template and its analytical applications

Motoko Mukae[a]; Toshihiro Ihara[a]; Miyuki Tabara[a]; Pelin Arslan[a]; Akinori Jyo[a]
[a] Department of Applied Chemistry and Biochemistry, Graduate School of Science and Technology, Kumamoto University, Kumamoto, Japan

To cite this Article Mukae, Motoko , Ihara, Toshihiro , Tabara, Miyuki , Arslan, Pelin and Jyo, Akinori(2009) 'Photodimerisation of anthracenes using a DNA template and its analytical applications', Supramolecular Chemistry, 21: 3, 292 — 295
To link to this Article: DOI: 10.1080/10610270802516625
URL: http://dx.doi.org/10.1080/10610270802516625

Supramolecular Chemistry
Vol. 21, Nos. 3–4, April–June 2009, 292–295

Taylor & Francis
Taylor & Francis Group

Photodimerisation of anthracenes using a DNA template and its analytical applications

Motoko Mukae, Toshihiro Ihara*, Miyuki Tabara, Pelin Arslan and Akinori Jyo

Department of Applied Chemistry and Biochemistry, Graduate School of Science and Technology, Kumamoto University, Kumamoto, Japan

(*Received 11 July 2008; final version received 28 September 2008*)

We tethered an anthracene group to one end of oligonucleotide to make anthracene–ODN conjugates (**5′AntODN15** and **3′AntODN25**). The sequences of the conjugates were designed to hybridise to adjacent sites of the template with their anthracene units stacked each other. The conjugates dimerised only in the presence of the template by light irradiation. The dimerisation efficiency was affected by one-base mismatch in the tandem duplexes. Furthermore, we studied the signal amplification experiments under thermal cycling in the presence of the targets with full match and mismatch sequences.

Keywords: DNA conjugate; chemical ligation; photodimerisation; anthracene; SNP analysis

Introduction

Single nucleotide polymorphisms (SNPs) represent the most abundant forms of DNA sequence variation in the human genome and are responsible for phenotypic diversity, influencing an individual's anthropometric characteristics, risk of certain disease, and variable response to drugs and the environment. Because of their dense distribution across the genome, SNPs are used as markers in medical diagnosis and personalised medicines. Several techniques for SNP genotyping based on the specificity of ligation using T4 and Tth ligases have been reported (*1*, *2*). Ligase is the enzyme that links two DNA fragments hybridised tandem on a complementary target. It recognises that one base mismatches strictly. Although the enzymatic ligation is very useful as the fundamental technique for genotyping, it should be carried out under physiological conditions.

Ligated products, however, do not necessarily need to have the structure of natural nucleotides in the applications of gene diagnoses *in vitro*. In the last decade, a number of laboratories have been in the quest of alternative reactions to enzymatic ligation and several chemical ligations, non-enzymatic linking of DNA ends, have been proposed mainly for DNA or RNA analysis (*3–5*). Among the chemical ligations reported so far, there have been a few reports concerning the ligations based on photochemical reactions (*6–8*). Photochemical ligation has several advantages, including the lack of a need for additives, low cost, and ease of reaction control by tuning wavelength, light strength and irradiation time.

Previously, we synthesised anthracene–DNA conjugates by covalently linking anthracenes to one end of oligonucleotides and demonstrated the photochemical ligation of the conjugates through dimerisation of anthracenes (*9*, *10*). It is well known that two anthracenes form (4 + 4) a photoadduct by light irradiation at 366 nm (Figure 1) (*11–13*). The conjugates dimerised only in the presence of the template by light irradiation. We found that the ligation yield depended on the substituted positions in anthracenes from where they were tethered to the end of DNA (*10*). The ligation yields of the conjugates with 1- and 2-substituted anthracenes were remarkably high compared with those with a 9-substituted one. The reactions of these photoligations are almost complete within 1 min of irradiation. Here, we examined the effect of the one base mismatch in the duplexes and the signal amplification under thermal cycling conditions.

Results and discussions

We synthesised a pair of anthracene–ODN (oligodeoxyribonucleotide) conjugates, **5′AntODN15** and **3′AntODN25**, which carry 1-substituted anthracenes on 5′ and 3′ ends of 15 mer and 25 mer ODNs, respectively. They were prepared by the coupling reactions between activated esters of anthracene and amino groups modified on 3′ or 5′ terminus of the ODNs. The sequences of the conjugates are designed to be complementary to the neighbouring sites of the template ODN (target) with their anthracene units facing each other. The conjugates were isolated using reversed phase HPLC and identified with MALDI-TOF/MS. All ODNs used in this study are shown in Figure 2.

To evaluate the thermal stabilities of the duplexes with the conjugates, UV thermal melting experiments were performed using UV–vis spectrophotometer equipped with Peltier temperature controller. The temperatures

*Corresponding author. Email: toshi@chem.kumamoto-u.ac.jp

ISSN 1061-0278 print/ISSN 1029-0478 online

DOI: 10.1080/10610270802516625
http://www.informaworld.com

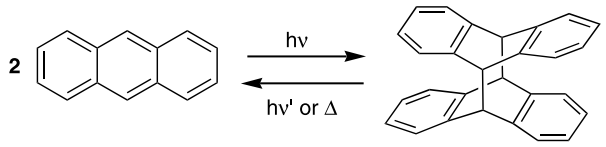
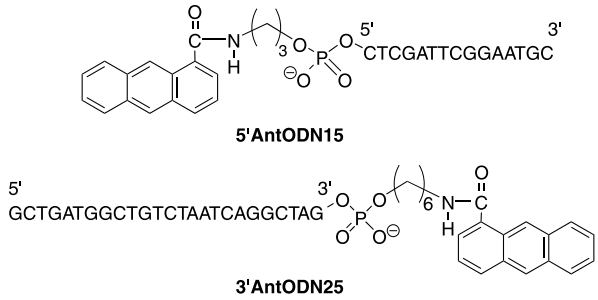


Figure 1. Photodimerisation of two anthracenes.

at which 50% of ODN components exist as a single strand were defined as melting temperatures, T_m, and used as an indicator of thermal stabilities of the duplexes. The obtained T_m values are summarised in Table 1. T_m values of the half-covered duplexes, **5′AntODN15/WT** and **3′AntODN25/WT,** are higher than those of the corresponding unmodified control duplexes, **ODN15/WT** and **ODN25/WT** ($\Delta T_m = 2.5, 1.9$). This stabilisation would be due to the interactions of anthracene units such as intercalation with the duplex of themselves or stacking with unpaired bases on **WT**. On the other hand, T_m value of tandem duplex, **5′AntODN15/3′AntODN25/WT**, was approximately equal to the unmodified control duplex, **ODN15/ODN25/WT**. Fluorescence studies on the tandem duplex, **5′AntODN15/3′AntODN25/WT**, indicated a weak excimer band of anthracenes, probably, due to a partial stacking of the two anthracene units of in the middle of the structure (data not shown). The free energy that came from this weak stacking interaction of anthracenes might be cancelled by the steric hindrance between the linker chains of neighbouring conjugates. This result inspired us to apply this system to SNP discrimination based on the local structural disruption induced by mispairing around the central part of the tandem duplex, because the subtle perturbation in local duplex structure would make the weakly stacked anthracene pair to change its static and/or dynamic relative geometry.



ODN15: 5' CTCGATTCGGAATGC 3'
ODN25: 5' GCTGATGGCTGTCTAATCAGGCTAG 3'
WT: 5' GCATTCCGAATCGAGCTAGCCTGATTAGACAGCCATCAGC 3'
Mut 1C: 5' GCATTCCGAATCGA**C**CTAGCCTGATTAGACAGCCATCAGC 3'
Mut 2T: 5' GCATTCCGAATCG**T**GCTAGCCTGATTAGACAGCCATCAGC 3'

Figure 2. Structures and sequences of anthracene–ODN conjugates and other ODNs used in this study.

Table 1. Melting temperatures of the duplexes[a].

Duplex	T_m/°C
ODN15/WT	40.0
ODN25/WT	51.9
ODN15/ODN25/WT	44.9, 52.3
5′AntODN15/WT	42.5
3′AntODN25/WT	53.8
5′AntODN15/3′AntODN25/WT	44.8, 53.9
5′AntODN15/3′AntODN25/Mut 1C	39.7, 54.3
5′AntODN15/3′AntODN25/Mut 2T	41.6, 53.9

[a] Conditions: 1 μM in each DNA strand, in a 10 mM phosphate buffer containing 1 mM EDTA (pH 7.0).

The tandem duplexes **5′AntODN15/3′AntODN25/WT** were irradiated at 366 nm for 1 min with a high-pressure Hg lamp. After irradiation, the reaction mixtures were analysed by RP-HPLC. The chromatograms showed the appearance of a new peak along with the disappearance of the **5′AntODN15** and **3′AntODN25** peaks (Figure 3). MALDI-TOF/MS indicated that the new peak obtained here had the *m/z* of the ligated product from **5′AntODN15** and **3′AntODN25**. This peak did not appear in the absence

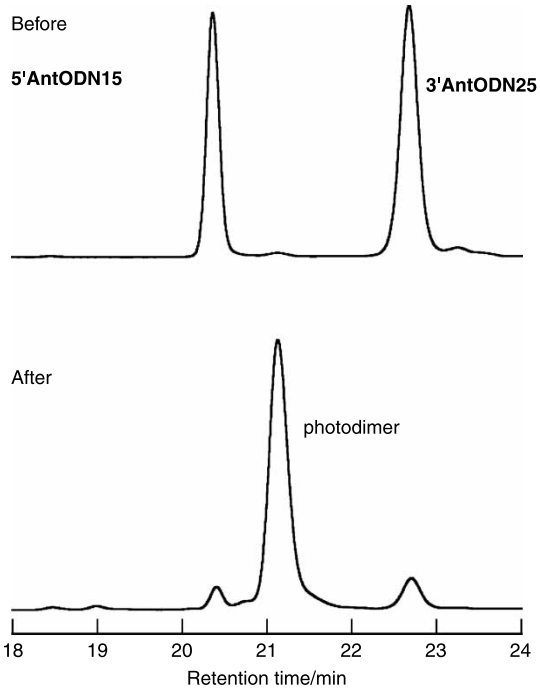


Figure 3. HPLC chromatograms of the reaction mixture, **5′AntODN15/3′AntODN25/WT**, before (top) and after 1 min photoirradiation (bottom). The solution containing 30 μM ODN in each strand, 10 mM phosphate buffer and 1 mM EDTA was irradiated in an Ar atmosphere (0°C) at 366 nm using a high-pressure mercury lamp equipped with appropriate optical glass filters. The chromatograms were obtained under the following conditions. Column, Wakosil-II 5C18 RS; solution A, 0.1 M TEAA buffer (pH 7); solution B, acetonitrile; linear gradient 5–30% in 30 min; flow rate, 1 ml/min.

of the template, **WT**. The 1:1 mixture of the product and **WT** showed the clear melting with the T_m at 56.8°C. This was much higher than that of the tandem duplex with the conjugates and lower than that of normal 40 mer duplex with the same sequence (61.3°C, data not shown). While the ligation converts the two short conjugates to one longer photodimer, which should show higher T_m, the bulky anthracene dimer located in the middle of the structure seems not to be favourable to form a duplex.

The ligation efficiencies were examined for several tandem duplexes with mismatched templates (**Mut1C**, **Mut2T**). The relative ligation yields after 1 min of irradiation are shown in Figure 4. The ligation efficiencies for the duplexes with the mismatched templates, **Mut1C** and **Mut2T**, were significantly low compared with those with the full matched template, **WT**. All photoirradiations were performed at 0°C, where the conjugates formed tandem duplexes regardless of the kinds of the templates. Therefore, the difference in the ligation yields observed here should be attributed to the local structural disruption at a mismatched base pair in the duplexes. Especially, the ligation for the duplex with **Mut2T** was significantly suppressed. Although it is apparent that this is due to the effect of the local structure perturbed by T–T mispairing at second position, comprehensive studies should be required to draw a complete picture of the effect of the mispairings and their positions on the yields of the photochemical ligation. Reaction time in the conventional enzymatic ligation protocols is typically 1–2 h (*1*, *2*). In contrast, photochemical ligation between anthracene and ODN conjugates only takes a minute. This could substantially reduce the time required for analyses.

We tried to amplify the signal by turning over the ligation reaction under thermal cycling conditions. As shown in Figure 5, cross-linked products could be amplified by cycling the conditions to promote hybridisation, dimerisation and denaturation. In this experiment, one cycle consists of the three steps, hybridisation at 0°C for 1 min, irradiation at 0°C for 1 min and denaturation at 80°C for 2 min. The results of the amplification experiment are shown in Figure 6. The initial concentration of the conjugates was 30 μM and that of the template, **WT**, 0.6 μM; the feeding ratio, [conjugate]/[**WT**], was 50. The amount of the photodimer was amplified with the number of thermal cycles. After 10 cycles, signal amplification factor (f_{amp}) reached 4.7-fold. In contrast, the reaction did not turn over without the denaturation step ($f_{amp} = 0.93$). T_m of the duplex with dimerised product (56.8°C) is much higher than that of the tandem duplex with the conjugates (44.8 and 53.9°C) as mentioned before. Therefore, it is natural that the reaction saturates before depletion of the conjugates because of product inhibition.

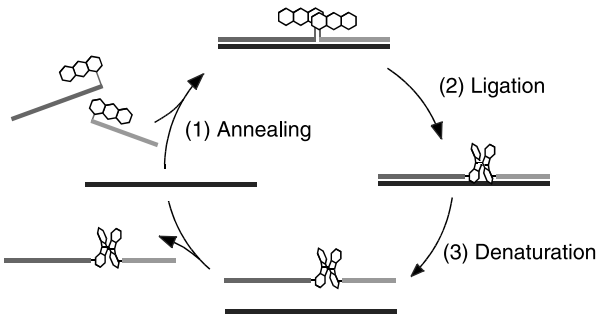


Figure 5. Asymmetric signal amplification system. Two probes (1) hybridise to adjacent sites of a nucleic acid target. Next, (2) irradiation covalently connects the two probes, and then the sample is heated to (3) denature the photodimer/target duplex and free the target for the next cycle.

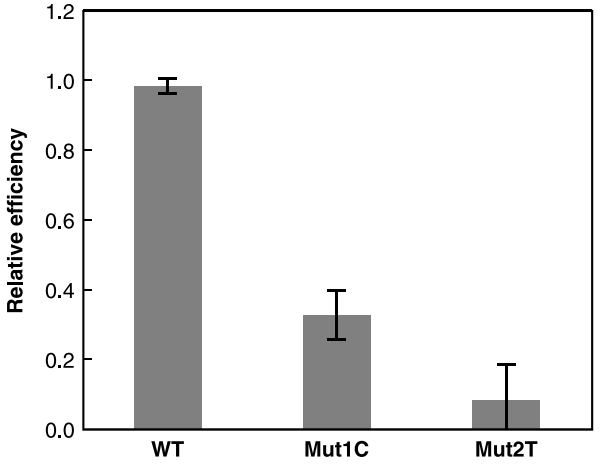


Figure 4. Ligation yields using several targets having a mismatch base at different positions. Conditions: 30 μM in each DNA strand, in a 10 mM phosphate buffer containing 1 mM EDTA (pH 7.0).

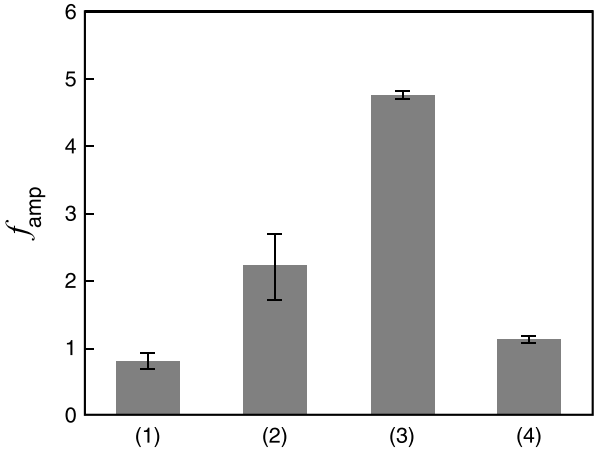


Figure 6. Signal amplification factors (f_{amp} = [photodimer]/[**WT**]) as a function of thermal cycling. (1) One cycle; (2) 2 cycles; (3) 10 cycles; (4) tandem duplex with **Mut 2T**; 10 cycles. Conditions: 30 μM **5AntODN15** and **3AntODN25**, 0.6 μM **WT** (**Mut 2T**), in a 10 mM phosphate buffer containing 1 mM EDTA (pH 7.0).

That is, the conjugates have to compete with the photodimer (product) for hybridisation with **WT**. They can hybridise only in the period when their amount is much higher than that of the photoproducts. f_{amp} will be improved with the reactions of higher feeding ratio. The amplification was performed for the tandem duplex with mismatched templates (**Mut 2T**). After 10 cycles, f_{amp} was almost at the same level of the signal as the one cycle ligation of tandem duplex with **WT**.

Conclusions

We demonstrated the photochemical ligation between anthracene and DNA conjugates. This reaction proceeded to more than 90% yield within 1 min. The ligation efficiency was affected by one-base displacements in the template sequence. This would be due to the local structural disruption around the mispairing in the duplexes. Amplification of ligated product was shown under thermal cycling. The result shows that the photochemical ligation between anthracene and DNA sconjugates could be applied for SNP detection, although the conditions need further optimisation. The combination with other techniques such as fluorometry or LC/MS would significantly improve the detection limit.

Acknowledgements

This work was partially supported by a grant-in-aid for JSPS fellow (18809000) (M.M) and for Scientific Research (B) (20350038) from JSPS (T.I.). The authors are also grateful to the support from PRESTO, JST (T.I.).

References

(1) Landegren, U.; Kaiser, R.; Sanders, J.; Hood, L. *Science* **1988**, *241*, 1077–1080.
(2) Barany, F. *Proc. Natl. Acad. Sci. USA* **1991**, *88*, 189–193.
(3) Xu, Y.; Karalkar, N.B.; Kool, E.T. *Nat. Biotechnol.* **2001**, *19*, 148–152.
(4) Ogasawara, S.; Fujimoto, K. *Angew. Chem. Int. Ed.* **2006**, *45*, 45102–4515.
(5) Silverman, A.P.; Kool, E.T. *Chem. Rev.* **2006**, *106*, 3775–3789.
(6) Liu, J.; Taylor, J.S. *Nucleic Acids Res.* **1998**, *26*, 3300–3304.
(7) Albagli, D.; Atta, R.V.; Cheng, P.; Huan, B.; Wood, M.L. *J. Am. Chem. Soc.* **1999**, *121*, 6954–6955.
(8) Fujimoto, K.; Matsuda, S.; Takahashi, N.; Saito, I. *J. Am. Chem. Soc.* **2000**, *122*, 5646–5647.
(9) Ihara, T.; Fujii, T.; Mukae, M.; Kitamura, Y.; Jyo, A. *J. Am. Chem. Soc.* **2004**, *126*, 8880–8881.
(10) Mukae, M.; Tabara, M.; Arslan, P.; Ihara, T.; Jyo, A. *Nucleic Acids Symp. Ser.* **2007**, *51*, 287–288.
(11) Bouas-Laurent, H.; Castellan, A.; Desvergne, J.P.; Lapouyde, R. *Chem. Soc. Rev.* **2000**, *29*, 43–55.
(12) Breton, G.W.; Yang, X. *J. Chem. Ed.* **1998**, *75*, 81.
(13) Becker, H.-D. *Chem. Rev.* **1993**, *93*, 145–172.