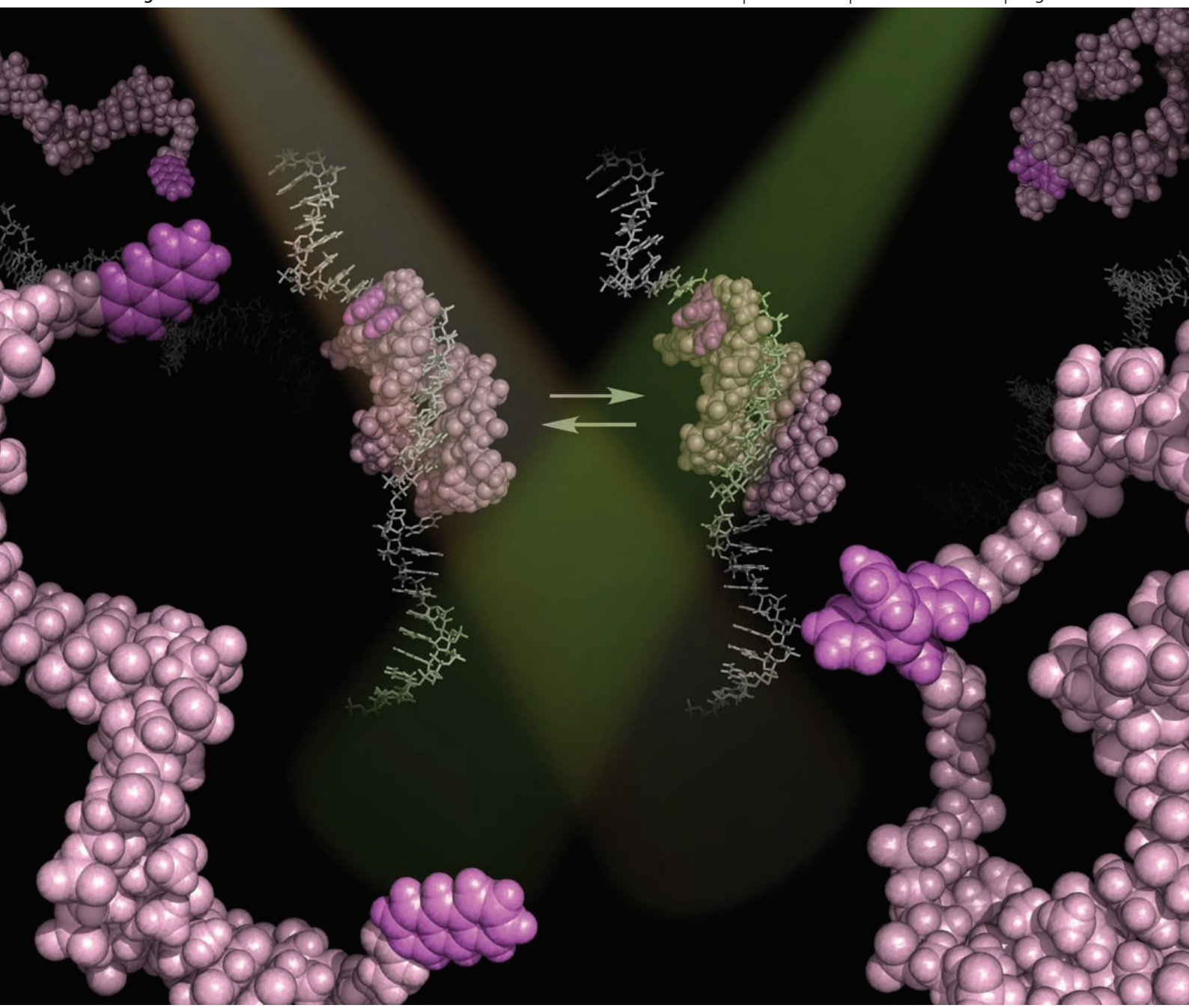
Organic & Biomolecular Chemistry

www.rsc.org/obc

Volume 8 | Number 21 | 7 November 2010 | Pages 4777–5020



ISSN 1477-0520

RSC Publishing

FULL PAPER

Toshihiro Ihara *et al.*

Reversible circularization of an anthracene-modified DNA conjugate through bimolecular triplex formation and its analytical application

Reversible circularization of an anthracene-modified DNA conjugate through bimolecular triplex formation and its analytical application†

Pelin Arslan,[a] Akinori Jyo[a] and Toshihiro Ihara*[a,b]

Received 18th June 2010, Accepted 21st July 2010
DOI: 10.1039/c0ob00282h

We prepared an oligodeoxyribonucleotide conjugate (**5-3ant$_2$18**) carrying two anthracenes, each of which was tethered to both ends of the conjugate through hexamethylene linker chains. The conjugate has a mirror repeat of two heptamer sequences, such that it forms a bimolecular triplex with the single stranded target, forming a two-fold U-shaped conformation. The conformation of the conjugate in its triplex structure could be frozen instantaneously by circularization through photodimerization of the anthracenes. Compared with the duplex formation of linear probes with relevant sequences, bimolecular triplex formation of **5-3ant$_2$18** shows a unique feature in its target recognition; it binds the target tightly, yet still retains high sequence selectivity. Circularization of **5-3ant$_2$18** by UV photoirradiation was verified as the probe reaction for a DNA assay. The probe reaction could be performed in a few seconds over a wide range of temperatures, at least between 0 and 25 °C. In addition, the reaction could be regarded as a reversible method for the preparation of circular DNA that shows higher affinity for the target.

Introduction

The DNA triple helix has attracted great attention due to its significant potential in nucleic acid sensing and relevance to gene function *in vivo*.[1] Triplex formation requires sequence specific recognition of the purine strand of the duplex by the third strand in the major groove of the duplex. There are two triplex types called pyrimidine (Py.PuPy) and purine (Pu.PuPy) motifs that are defined by the orientation of the third strand (Pu and Py stand for purine and pyrimidine strands, respectively). In the Py.PuPy motif, the third strand (Py) binds in a parallel orientation to the Pu strand of the duplex through Hoogsteen hydrogen bonding, whereas in Pu.PuPy, the orientation of the third strand (Pu) is antiparallel to the Pu strand and it binds through reverse-Hoogsteen pairing.[2]

The two Py strands in a Py.PuPy motif connected by a loop form a stable bimolecular triplex with a complementary purine-rich single strand.[3] The structure is also observed *in vivo* as the body of H-DNA.[4] The Py strand captures the Pu strand very tightly by cooperative formation of Watson–Crick and Hoogsteen hydrogen bonding (entropic effect). The selectivity is high compared with the recognition through duplex formation, because the number of hydrogen bonds per nucleotide is more than that in the duplex. The G and A bases in the Pu strand form five and four hydrogen bonds, which are convergently oriented to the bases from the two sides in the preorganized cleft of the hairpin-shaped Py strand.

DNA probing techniques generally rely on the specificity of the target recognition through complementary duplex formation. While longer probes are desirable for sensitive detection due to their tight binding to the target, they are somewhat tolerant to one-base mismatches. Although shorter probes have a higher specificity, their affinity is not sufficient to be used in samples with low target concentration. This dilemma would be alleviated by the recognition of the target single strand through bimolecular triplex formation. The hairpin-shaped Py strand binds to the shorter target with higher stability. It would strike a balance between selectivity and sensitivity.

The entropic benefit derived from preorganization would become more pronounced if other ends of the hairpin were covalently connected with each other to make it circular. In addition, circularization would confer resistance to exonuclease degradation. Considering these properties, synthesis of circular oligonucleotides has been under investigation both by enzymatic and non-enzymatic approaches.[5]

In the present study, we demonstrated quick reversible photocircularization of an anthracene-modified oligodeoxyribonucleotide (ODN) conjugate, **5-3ant$_2$18**, and provided an example of its analytical application. **5-3ant$_2$18** is an 18-mer ODN with a mirror repeat of two heptamer sequences linked to each other with a four nucleotide loop and carries two anthracenes, one on each end. **5-3ant$_2$18** forms a bimolecular triplex with the Pu strand with both its anthracene units facing each other as shown in Fig. 1(a). Two anthracenes are known to form a dimer by a [4π-4π] photoreaction (Fig. 2).[6] We have already shown that this reaction effectively proceeds on the various DNA scaffolds and would be useful as a method of photochemical ligation for DNA analyses.[7] Undesired crosslinking reactions with nucleobases was not observed, because the photoreaction involving anthracene is orthogonal to the typical [2π+2π] reaction that nucleobases take part in. **5-3ant$_2$18** is thought to circularize in the presence of the template (target) Pu strand (**fm7**) by photoirradiation at 366 nm and return to its linear form under light with a shorter wavelength. The unique thermodynamic feature of bimolecular triplex formation was applied to the analysis of a single

[a]*Department of Applied Chemistry and Biochemistry, Graduate School of Science and Technology, Kumamoto University, 2-39-1 Kurokami, Kumamoto 860-8555, Japan. E-mail: toshi@chem.kumamoto-u.ac.jp; Fax: +81-96-342-3873; Tel: +81-96-342-3873*
[b]*PRESTO, Japan Science and Technology Agency, San-bancho Building, 3-5 Sanbancho, Chiyoda-ku, Tokyo 332-0012, Japan*

† Electronic supplementary information (ESI) available: Melting curves of the tandem duplexes and circular product are included. See DOI: 10.1039/c0ob00282h

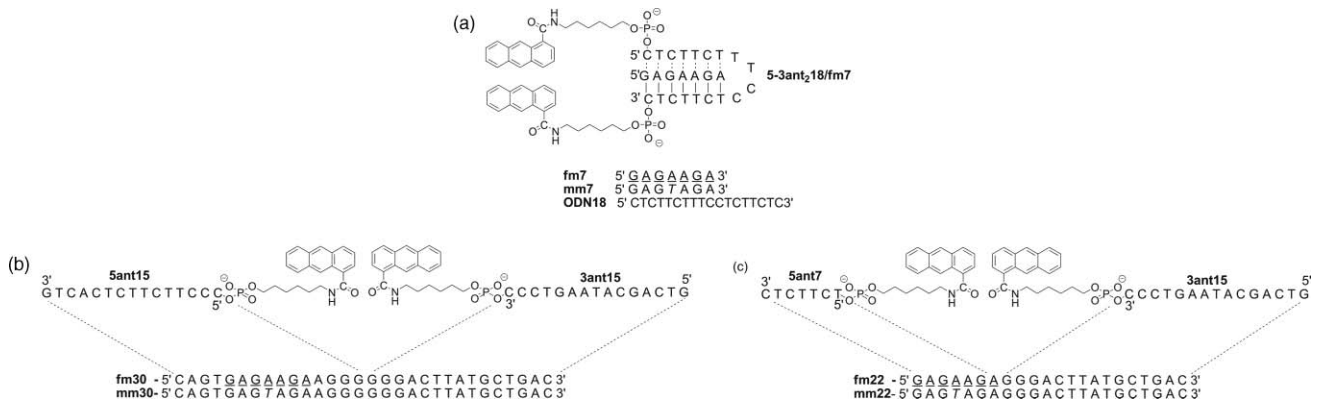


Fig. 1 Sequences and structures of ODNs and anthracene-ODN conjugates used in the present study. (a) Bimolecular triplex consisting of **5-3ant$_2$18**, and **fm7** or **mm7**; (b) long duplex consisting of **5ant15**, **3ant15** and **fm30** or **mm30**; (c) short duplex consisting of **5ant7**, **3ant15** and **fm22** or **mm22**. Mispaired bases are shown in italics.

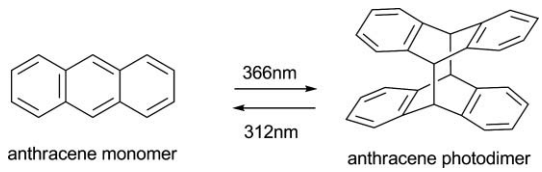


Fig. 2 Reversible photodimerization of anthracenes.

stranded target by taking advantage of the photocircularization of **5-3ant$_2$18**.

Results and discussion

Design of the systems

Fig. 1 shows the sequences and structures of ODNs and the anthracene-ODN conjugates used in this study. **5-3ant$_2$18** is an ODN conjugate that is doubly modified with anthracene on both ends and consisted of two mirror repeat heptamer sequences connected by a four nucleotide loop. A pyrimidine motif was employed in designing the conjugate as shown in Fig. 1(a). The heptamer sequence of the 3′ side forms a duplex with the fullmatch target, **fm7**, through Watson–Crick base pairing, and the other heptamer of the 5′ side folds back to the major groove of the duplex, as the third strand, to form Hoogsteen base pairs in the parallel orientation to **fm7**. **mm7** is the heptamer target in which the central A base in **fm7** is replaced by T. To verify the potential of bimolecular triplex formation as a recognition mode in DNA probing, two tandem duplex systems were designed as references. Each duplex consisted of two anthracene-ODN conjugates and a target. The sequences of the two conjugates were designed such that they hybridized to adjacent sites on the target with their anthracene units facing each other. The longer duplexes consisted of two 15-mer probes, **5ant15** and **3ant15**, and a 30-mer target that contains the sequence of **fm7** or **mm7** in the recognition site. The fullmatch duplex, **5ant15/fm30/3ant15**, was designed to give similar melting temperature (T_m) to **5-3ant$_2$18/fm7** under the experimental conditions used. The shorter duplexes consisted of the two conjugates, **5ant7** and **3ant15**, and a 22-mer target in which the heptamer recognition sequence is identical to that of **5-3ant$_2$18**. That is, the former duplex with fullmatch target had a similar thermal stability with **5-3ant$_2$18/fm7** for longer recognition site, the latter was supposed to show reduced stability compared with **5-3ant$_2$18/fm7** because of its duplex structure.

All the ODN conjugates were prepared by post modification. The synthesis of the singly modified conjugates, **5ant7**, **5ant15**, and **3ant15** was carried out according to a previously shown method.[7] **5-3ant$_2$18** was obtained by a one-step reaction, which was essentially the same as that used for the other singly modified conjugates, in moderate yield.

Thermal stability of the bimolecular triplex

Melting studies were performed to evaluate the effect of the anthracene moieties and mispairing on the stability of the bimolecular triplex. Experiments were carried out in 100 mM AcOH/AcONa buffer solution (pH 5.0, 10 mM NaCl, 10 mM $MgCl_2$). The melting curves of all triplexes were typical monophasic, showing that both base-pairings (Watson–Crick and Hoogsteen) melted cooperatively (Fig. 3).[8] The melting temperatures of the bimolecular triplexes and the control duplexes are shown in Table 1. Modification with an anthracene group on each end of the 18-mer ODN significantly stabilized the structure

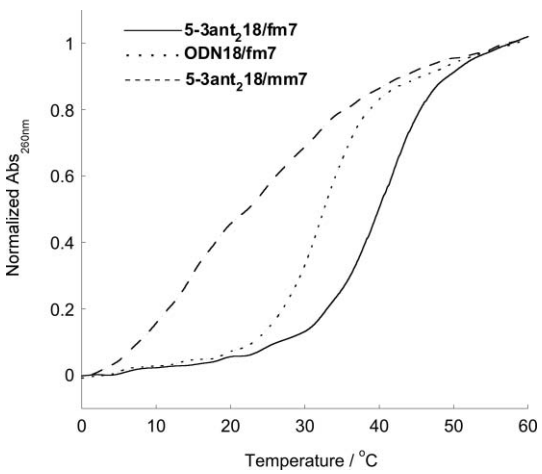


Fig. 3 Normalized melting curves of the bimolecular triplexes. Dotted, solid, and broken curves show the melting profiles of the **ODN18/fm7**, **5-3ant$_2$18/fm7** and **5-3ant$_2$18/mm7**, respectively.

Table 1 Melting temperatures of the bimolecular triplexes and control duplexes[a]

	DNA complex	T_m/°C	ΔT_m/°C
Bimolecular triplexes	**5-3ant$_2$18/fm7**	42.1	
	5-3ant$_2$18/mm7	14.9	27.2[b]
	ODN18/fm7	32.4	9.7[b]
	ant∧ant18c/fm7	46.6	− 4.5[b]
Tandem duplexes	**5ant15/fm30/3ant15**	45.1	
	5ant15/mm30/3ant15	35.6, 48.2	9.5[c]
	5ant7/fm22/3ant15	~9[d], 49.8	
	5ant7/mm22/3ant15	—[e], 49.4	> ~9[d]

[a] The temperatures that gave the maximum first derivative of the melting curves were shown as the T_m. [b] The difference with the T_m of **5-3ant$_2$18/fm7**. [c] The difference of the lower T_m (**5ant15**) with that of **5ant15/fm30/3ant15**. [d] **5ant7** dissociated first at lower temperature. T_m was not determined precisely, because the lower baseline was not observed clearly. [e] The inflection was not observed in the melting curve at lower temperature region.

of the bimolecular triplex compared with unmodified 18-mer ODN (**ODN18**). The difference in the melting temperature, ΔT_m, between **5-3ant$_2$18/fm7** and **ODN18/fm7** was 9.7 °C. This difference in thermal stability was most likely due to the influence of the π–π stacking between the anthracene units. This favorable feature formed the basis for the rest of this work, because the two anthracenes need to overlap to enable their dimerization.

The sequence selectivity of bimolecular triplex formation was also examined by UV melting experiments. The length of the longer duplex, **5ant15/fm30/3ant15**, was adjusted such that its T_m would be similar to that of bimolecular triplex **5-3ant$_2$18/fm7** under the experimental conditions. The melting profile of **5ant15/fm30/3ant15** showed a monophasic behaviour. The apparent T_m value of this complex was 45.1 °C. However, the longer duplex with mismatch target (**5ant15/mm30/3ant15**) showed biphasic melting profile where the **5ant15** dissociated from **mm30** before **3ant15**. One T-T mispairing in the longer duplex decreased the T_m by 9.5 °C. On the other hand, the T_m value of the bimolecular triplex decreased by 27.2 °C by the introduction of one T-T mispairing at the central position (**5-3ant$_2$18/mm7**). As expected, the selectivity in the formation of the bimolecular triplex was much higher than that of the duplex with similar thermal stability.

Circularization of **5-3ant$_2$18** through anthracene dimer formation could be used to probe its binding with the targets, because the folded shape of **5-3ant$_2$18** in the triplex **5-3ant$_2$18/fm7** would be topologically locked as the circular form instantaneously by the photodimerization of anthracenes. The circular form of **5-3ant$_2$18** was easily differentiated from its linear form by techniques such as HPLC or gel electrophoresis. The large difference in thermal stability (ΔT_m) of the complexes with fullmatch and mismatch targets observed here provided a wider range of temperatures under which irradiation experiments could be performed.

Photocircularization and its reversibility

Photoirradiation experiments were performed for **5-3ant$_2$18** in the presence of **fm7**. Fig. 4 shows the reversed phase HPLC (RP-HPLC) chromatograms of the reaction mixtures before and after light irradiation (LED lamp, λ = 366 nm, 0 °C, 3 s). The photoreactions were carried out for the solution of 2.2 μM

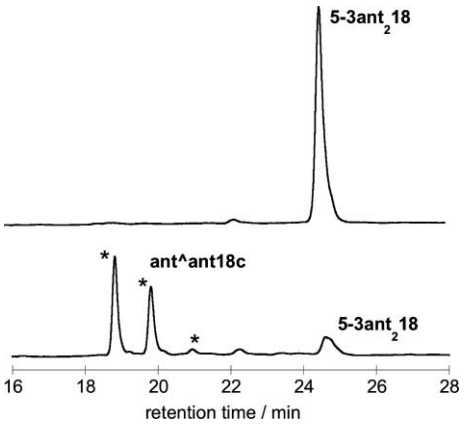


Fig. 4 RP-HPLC chromatograms of the reaction mixtures before (top) and after (bottom) 3 s irradiation for **5-3ant$_2$18/fm7**. The chromatograms were obtained under the following conditions: Column, Inertsil ODS-3; solution A, 0.1 M triethylammonium acetate buffer (TEAA buffer) (pH 7.0); solution B, acetonitrile; linear gradient, 5–45% in 30 min; flow rate, 1 ml min^{-1}. * indicates the new peaks that appeared after irradiation.

5-3ant$_2$18 and 3.3 μM **fm7** in 100 mM AcOH/AcONa buffer (pH 5.0, 10 mM NaCl and 10 mM $MgCl_2$). After 3 s of irradiation, the appearance of new peaks along with the disappearance of the conjugate was observed. Time profiles of the reaction are shown in Fig. 5(a). No change was observed in the chromatogram in the absence of the template (data not shown). These new peaks were identified by MALDI-TOF MS and confirmed to have identical mass with **5-3ant$_2$18**. Therefore, they could be attributed to the circularized form of the conjugate, **ant∧ant18c**. Several peaks with the same mass observed after irradiation could be the isomeric structures of the anthracene dimer moiety in **ant∧ant18c**, because up to eight isomers, including two pairs of enantiomers, could be generated in dimer formation.

Circularization of the conjugate was also confirmed by UV melting experiments. The bimolecular triplex of the circular conjugate with **fm7**, **ant∧ant18c/fm7**, showed higher thermal stability than that of the **5-3ant$_2$18/fm7** by 4.5 °C. This enhancement in stability could be attributed to a further entropic gain from circularization. That is, circularization of the conjugate favorably contributed to preorganization of the triplex structure.[5]

The reversibility of the reaction was examined by light irradiation with a shorter wavelength (transilluminator, λ = 312 nm, 0 °C) to **ant∧ant18c**. After 1 h of irradiation at 312 nm, *ca.* 80% of **ant∧ant18c** isomers reverted to **5-3ant$_2$18**. The reaction profile is shown in Fig. 5(b). Our doubly anthracene-tethered conjugate was prepared by a simple one-step reaction. Its circularization is quick, reversible, and generates a high yield. Therefore, the present method would be a convenient technique for the preparation of circular DNA ligands with high affinity to single stranded targets.

Sequence selectivity of photochemical ligation

Sequence selectivity in the ligation (circularization) of the bimolecular triplex was studied by comparing the yields with those of the duplexes. As mentioned before, two tandem duplex systems were designed as references. The long tandem duplexes (**5ant15**/target/**3ant15**) had almost the same thermal stability as the bimolecular triplex for their fully matched complexes under

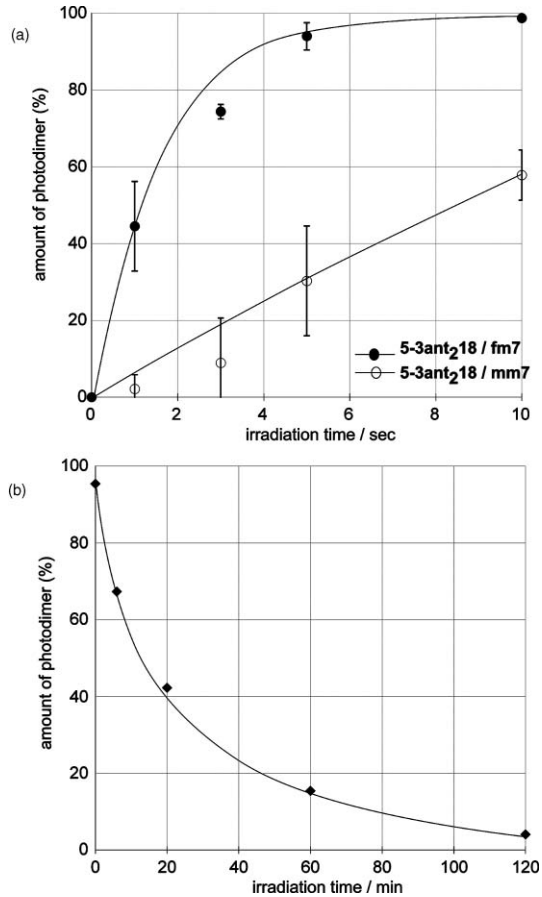


Fig. 5 Time courses of photoreactions. (a) The photoligation (circularization) of bimolecular triplexes in the presence of the fullmatch (**fm7**) and mismatch (**mm7**) targets. Reaction mixtures contained 3.3 μM of heptamer targets (**fm7** or **mm7**) and 2.2 μM of **5-3ant$_2$18** in 100 mM AcOH/AcONa buffer (pH 5.0, 10 mM NaCl, 10 mM $MgCl_2$). (b) Time course for the photodissociation of anthracene photodimer (**ant∧ant18c**) upon irradiation at 312 nm.

the present experimental conditions. The short tandem duplexes (**5ant7**/target/**3ant15**) had rather lower thermal stability than the bimolecular triplex, because its recognition sequence is as short as in the triplex system (7-mer).

Photoirradiation experiments were carried out for the bimolecular triplex and the two tandem duplexes. The time courses of the ligation were examined at 0 and 25 °C for each of the three systems with fullmatch and mismatch targets. The time courses obtained for **5-3ant$_2$18/fm7** and **5-3ant$_2$18/mm7** at 25 °C are shown in Fig. 5(a). This temperature is in between the T_ms of **5-3ant$_2$18/fm7** and **5-3ant$_2$18/mm7**. It is obvious that one mispairing at the centre of the heptamer target significantly affected the yields of the circularization.

Although both reactions seem to proceed even on mismatch triplex, **3ant$_2$18/mm7** in due course, we found significant differences in their initial reaction rates. The ligation yield after 3 s irradiation on **5-3ant$_2$18/fm7** was higher than that of **3ant$_2$18/mm7**, by almost 10-fold. Initial reaction rates obtained for all complexes are summarized in Fig. 6. The contrast in the reaction rates obtained at 0 °C were very high for the pair of short duplexes, **5ant7/fm22/3ant15** and **5ant7/mm22/3ant15**. The ligations scarcely proceeded at 25 °C for both duplexes, because

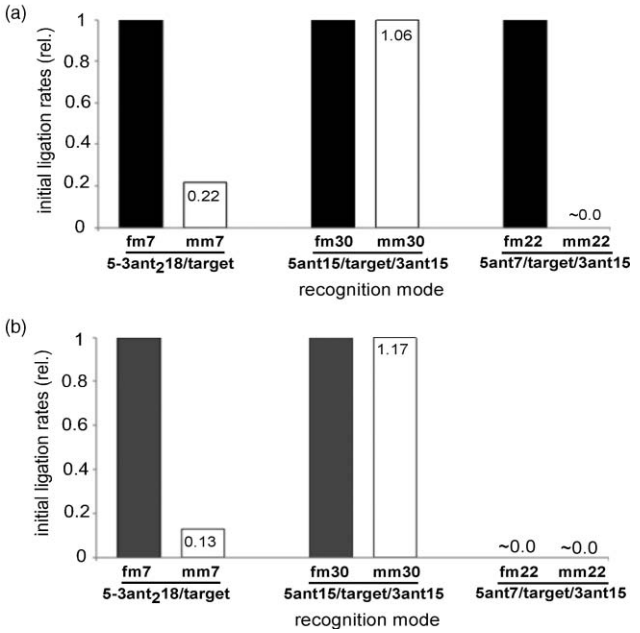


Fig. 6 Bar diagrams of relative initial ligation rates ($t \leq 3$ s) for bimolecular triplexes (**5-3ant$_2$18**/target), long duplexes (**5ant15**/target/**3ant15**), and short duplexes (**5ant7**/target/**3ant15**) containing fullmatch or mismatch targets for each system. Photoirradiation were performed at (a) 0 °C and (b) 25 °C.

5ant7 dissociates even from the fullmatch duplex with **fm22** (T_m = ~9 < 25 °C). In general, it is not difficult to optimize the conditions to generate high signal contrast in the case of short DNA probes.[7] The impact of one-base mismatch on thermal stability (ΔT_m: $T_{m,fm} - T_{m,mm}$) is larger for shorter probes (here the subscripts, "fm" and "mm" mean fullmatched and mismatched complexes, respectively). Only in between the T_ms of the complexes with fullmatched and mismatched targets, could an effective assay be carried out. However, the assay needed to be carried out at low temperature because of its lower thermal stability, which made it difficult for the short probes to be applied to the highly diluted sample solutions. Meanwhile, for the pair of long duplexes, the reaction rate on **5ant15/fm30/3ant15** was almost the same or slightly low compared with **5ant15/mm30/3ant15** at both temperatures. Although the thermal stability of **5ant15/fm30/3ant15** is almost the same as **5-3ant$_2$18/fm7**, **fm30** was not distinguished from **mm30**, because the difference in the stabilities of the complexes, **5ant15/fm30/3ant15** and **5ant15/mm30/3ant15**, was not enough to thermodynamically separate **fm30** from **mm30** at any temperature. The results show that **5-3ant$_2$18** binds to the target tightly, yet it still retains high sequence selectivity. That is, the formation of a bimolecular triplex by **5-3ant$_2$18** enjoys two inconsistent features, tight binding and high selectivity, in molecular recognition. There is another point that should be noted in Fig. 6. A moderate contrast in reaction rates was observed for **5-3ant$_2$18/fm7** and **5-3ant$_2$18/mm7**, even at 0 °C. This could not be explained only by the thermal stability of the ODN complexes, because even the T_m of **5-3ant$_2$18/mm7** (14.9 °C) is higher than 0 °C. It could be inferred that the local structural disruption or the dynamic conformational fluctuation at the central T.TT mispaired triplet propagates to the terminal moiety of the triplex thus affect the static or dynamic conformation around the anthracenes. This

was an unexpected favorable feature of this system. It allows us to carry out the assay (the photochemical ligation) over a wide range of temperatures. This has the advantage that the reaction temperature would not need to be strictly adjusted when designing multiplex assays.

Bimolecular triplex formation followed by photocircularization of **5-3ant$_2$18** would be the promising probe reaction for single stranded target DNAs under the present experimental conditions (pH 5.0). It is well known that the formation of C^+.GC triplets in pyrimidine motif of DNA triplex are pH dependent and weak acidic conditions are favored to make it easier for cytosines on the third strand to be protonated. In order to make our bimolecular triplex applicable in physiological pH, some modifications would be necessary. Base[9] and/or backbone[10] modifications should be effective for the improvement of the stability at neutral pH. Specific interactions of triplex structures with the additives such as small ligands[11] and metal ions[12] would also be very useful.

Conclusions

In this study, we reported a reversible circularization of ODN conjugate, **5-3ant$_2$18**, carrying an anthracene group on each end through hexamethylene linker chains. It formed a circular structure, **ant∧ant18c**, in 10 s by 366 nm irradiation in the presence of the fullmatched target and returned to the linear form under light at 312 nm. Uniquely, the bimolecular triplex formation of **5-3ant$_2$18** bound the target tightly, yet still retained high sequence selectivity. Circularization of **5-3ant$_2$18** would be useful in a DNA assay as the probe reaction, because the folded conformation of the conjugate is frozen instantaneously by the reaction. Preliminary studies showed that the reaction could be carried out in a few seconds over a wide range of temperatures. In addition, the reaction could be regarded as a reversible method for the preparation of circular DNA that shows higher affinity for its target.

Experimental

Reagents and equipment

The 18-mer ODN carrying two amino groups tethered at the 5′- and 3′-ends through hexamethylene linker chain (**5-3amino$_2$18**) were purchased from Operon Biotechnologies, Inc (USA) or synthesized according to the conventional procedure using an automated DNA synthesizer (Expedite 8900, Applied Biosystems). All reagents for ODN synthesis were of the highest grade commercially available and used without further purification. The 15-mer ODNs carrying 5′ or 3′- amino group (**5amino15** and **3amino15**) and the 7-mer ODN carrying a 5′-terminal amino group (**5amino7**) through a hexamethylene linker were purchased from Hokkaido System Science Co., Ltd. (Japan). These amino group-modified ODNs were all purified by RP-HPLC on an Inertsil ODS-3 column (4.6 × 150 mm, 5 μm particle size) or a Wakosil-II 5C18 column (4.6 × 150 mm, 5 μm particle size) with a linear gradient of acetonitrile in 0.1 M triethylammonium acetate buffer (TEAA buffer) (pH 7.0) and subsequently identified with MALDI-TOF mass spectrometry (Voyager RP, PerSeptive Biosystems). All the target ODNs (**fm7**, **mm7**, **fm30**, **mm30**, **fm22**, and **mm22**) were also purchased from Hokkaido System Science Co., Ltd. (Japan).

Synthesis of anthracene modified oligonucleotide conjugates

Anthracene-ODN conjugates (**5-3ant$_2$18**, **3ant15**, **5ant15**, and **5ant7**) were synthesized by the coupling of primary amino groups tethered on the 5′- and/or 3′-end with succinimidyl 1-anthracene carbonate. In a typical procedure, amino group-modified ODNs (30 nmol) (**5-3amino$_2$18**, **5amino15**, **3amino15**, and **5amino7**) were dissolved in 30 μL of 0.2 M Na_2CO_3/$NaHCO_3$ buffer (pH 9.35). To this solution was added 60 μL of a DMSO solution of succinimidyl 1-anthracene carbonate (25 mM, 1500 nmol). The suspended solution was sonicated for 10 min, and then stirred at room temperature overnight. The reaction mixture was diluted to 200 μL with 20% acetonitrile aqueous solution and subjected to a NAP10 column (Sephadex G-25, Amersham Biosciences). The obtained crude product was further purified by RP-HPLC with the same solution system used for purification of amino group-modified ODNs and then identified by MALDI-TOF MS.

Thermal denaturation experiments

Thermal denaturation experiments were carried out in AcOH/AcONa buffer solution (100 mM, pH 5.0, 10 mM NaCl, 10 mM $MgCl_2$). The concentration of each DNA component was 1 μM. All experiments were performed on a 1650pc UV/Vis Spectrophotometer equipped with a Peltier thermal controller (Shimadzu). Prior to the beginning of each denaturation experiment, the samples were degassed at 85 °C for 5 min and then annealed with slow cooling to 0 °C. After equilibration for 30 min at 0 °C, the solutions were heated slowly at a rate of 0.5 deg min^{-1} with blowing dry N_2 gas (only below room temperature) to the cuvette to prevent dew condensation. The melting profiles were monitored by absorption change at 260 nm. The temperature that gave the maximum first derivative of the melting curve was used as the T_m, a measure of the thermal stability of the triplex and the controls. Thermal denaturation experiments were repeated 2–3 times for each sample and the reported T_m values are the average of all duplicates or triplicates for a given sample.

Photoirradiation experiments

The anthracene-ODN conjugates (2.2 μM) were irradiated in the presence of their fullmatch or mismatch target (3.3 μM) in the same buffer solutions used in the melting experiments. Prior to irradiation, reaction mixtures were heated to 85 °C and then annealed with slow cooling to 0 °C at the rate of 1 deg min^{-1} on a heating block (TAITEC CTU-N). Reaction mixtures were irradiated in an Ar atmosphere with 1600 mW cm^{-2} Omron UV-LED (λ = 366 nm) at 0 °C or 25 °C. UVL8H was used as the lens unit with a working distance of 1.5 cm in each irradiation experiment. A transilluminator (λ = 312 nm, Vilber Lourmat UV transilluminator) was used for the reversed splitting reaction. The solution of circular photoproducts, **ant∧ant18c**, was irradiated at 0 °C. The products formed after photoirradiation were analyzed using RP-HPLC on an Inertsil ODS-3 column or a Unison UK C18 column with the same solution system used for ODN purification. Elution of the reaction products was monitored by absorption at 260 nm. The yield of cyclization and the reversed reaction was calculated from the peak area of the HPLC chromatograms after light irradiation. Photoirradiation

experiments were repeated 2–3 times for each sample and the reported yields are the average of all experiments.

Acknowledgements

This work was partially supported by a Grant-in-Aid for Scientific Research on Innovative Areas ("Coordinating Programming" Area 2107, No. 22108529) from MEXT (T.I.).

References

1 C. Hélène and J. J. Toulmé, *Biochim. Biophys. Acta, Gene Struct. Expression*, 1990, **1049**, 99–125; C. Hélène, *Pure Appl. Chem.*, 1994, **66**, 663–669; J.-S. Sun, T. Garestier and C. Hélène, *Curr. Opin. Struct. Biol.*, 1996, **6**, 327–333; G. M. Carbone, E. McGuffie, S. Napoli, C. E. Flanagan, C. Dembech, U. Negri, F. Arcamone, M. L. Capobianco and C. V. Catapano, *Nucleic Acids Res.*, 2004, **32**, 2396–2410; J. J. Bissler, *Front. Biosci.*, 2007, **12**, 4536–4546; A. Jain, G. Wang and K. M. Vasquez, *Biochimie*, 2008, **90**, 1117–1130; M. Duca, P. Vekhoff, K. Oussedik, L. Halby and P. B. Arimondo, *Nucleic Acids Res.*, 2008, **36**, 5123–5138; R. D. Wells, *FASEB J.*, 2008, **22**, 1625–1634.

2 V. N. Soyfer and V. N. Potaman, in *Triple Helical Nucleic Acids*, ed. R. C. Garber, Springer-Verlag, New York, 1996, ch.3, pp. 103–109.

3 L. E. Xodo, G. Manzini and F. Quadrifoglio, *Nucleic Acids Res.*, 1990, **18**, 3557–3564; C. Giovannangeli, T. M. Garestier, M. Rougée, M. Chassignol, N. T. Thuong and C. Hélène, *J. Am. Chem. Soc.*, 1991, **1049**, 99–125; M. Salunkhe, T. Wu and R. L. Letsinger, *J. Am. Chem. Soc.*, 1992, **114**, 8768–8772; M. A. Booher, S. Wang and E. T. Kool, *Biochemistry*, 1994, **33**, 4645–4651; S. Wang, M. A. Booher and E. T. Kool, *Biochemistry*, 1994, **33**, 4639–4644; E. Rubin, S. Rumney IV, S. Wang and E. T. Kool, *Nucleic Acids Res.*, 1995, **23**, 3547–3553; E. T. Kool, *Acc. Chem. Res.*, 1998, **31**, 502–510; A. A. Mundt, G. J. Crouch and B. E. Eaton, *Biochemistry*, 1997, **36**, 13004–13009; T. Ihara, Y. Sato, H. Shimada and A. Jyo, *Nucleosides, Nucleotides Nucleic Acids*, 2008, **27**, 1084–1096.

4 S. L. Broitman, *Prog. Biophys. Mol. Biol.*, 1995, **63**, 119–129; C. Giovannangeli and C. Hélène, *Antisense Nucleic Acid Drug Dev.*, 1997, **7**, 413–421; R. Zain and J.-S. Sun, *Cell. Mol. Life Sci.*, 2003, **60**, 862–870.

5 C. Giovannangeli, N. T. Thuong and C. Hélène, *Proc. Natl. Acad. Sci. U. S. A.*, 1993, **90**, 10013–10017; K. Ryan and E. T. Kool, *Chem. Biol.*, 1998, **5**, 59–67; A. V. Maksimenko, E. M. Volkov, J.-R. Bertrand, H. Porumb, C. Malvy, Z. A. Shabarova and M. B. Gottikh, *Eur. J. Biochem.*, 2003, **267**, 3592–3603; K. Fujimoto, S. Matsuda, Y. Yoshimura, T. Ami and I. Saito, *Chem. Commun.*, 2007, 2968–2970; R. Kumar, A. H. El-Sagheer, J. Tumpane, P. Lincoln, L. M. Wilhelmsson and T. Brown, *J. Am. Chem. Soc.*, 2007, **129**, 6859–6864; A. H. El-Sagheer, R. Kumar, S. Findlow, J. M. Werner, A. N. Lane and T. Brown, *ChemBioChem*, 2008, **9**, 50–52; P. Kocalka, A. H. El-Sagheer and T. Brown, *ChemBioChem*, 2008, **9**, 1280–1285.

6 H. Bouas-Laurent and J.-P. Desvergne, in *Photochromism Molecules and Systems*, ed. H. Durr and H. Bouas-Laurent, Elsevier, Amsterdam, 1990, ch.14, pp. 561–580; A. Gilbert, and J. Baggott, in *Essentials of Molecular Photochemistry*, 1991, CRC Press, Boca Raton.

7 T. Ihara, T. Fujii, M. Mukae, Y. Kitamura and A. Jyo, *J. Am. Chem. Soc.*, 2004, **126**, 8880–8881; P. Arslan, T. Ihara, M. Mukae and A. Jyo, *Anal. Sci.*, 2008, **24**, 173–176; M. Mukae, T. Ihara, M. Tabara and A. Jyo, *Org. Biomol. Chem.*, 2009, **7**, 1349–1354.

8 H.-T. Lee, S. Arciniegas and L. A. Marky, *J. Phys. Chem.*, 2008, **112**, 4833–4840; H.-T. Lee, I. Khutsishvili and L. A. Marky, *J. Phys. Chem.*, 2010, **114**, 541–548.

9 H. Li, V. J. Broughtan-Head, G. Peng, V. E. C. Powers, M. J. Ovens, K. R. Fox and T. Brown, *Bioconjugate Chem.*, 2006, **17**, 1561–1567.

10 S. M. Abdur Rahman, S. Seki, S. Obika, S. Haitani, K. Miyashita and T. Imanishi, *Angew. Chem., Int. Ed.*, 2007, **46**, 4306–4309; N. Kumar, K. E. Nielsen, S. Maiti and M. Peterson, *J. Am. Chem. Soc.*, 2006, **128**, 14–15.

11 G. C. Silver, J.-S. Sun, C. H. Nguyen, A. S. Boutorine, E. Bisagni and C. Hélène, *J. Am. Chem. Soc.*, 1997, **119**, 263–268; D. A. Gianolio, J. M. Segismundo and L. W. McLaughlin, *Nucleic Acids Res.*, 2000, **28**, 2128–2134; S. Basili, A. Bergen, F. Dall'Acqua, A. Faccio, A. Granzhan, H. Ihmels, S. Moro and G. Viola, *Biochemistry*, 2007, **46**, 12721–12736; A. Eick, F. Riechert-Krause and K. Weisz, *Bioconjugate Chem.*, 2010, **21**, 1105–1114.

12 T. Ihara, T. Ishii, N. Araki, A. W. Wilson and A. Jyo, *J. Am. Chem. Soc.*, 2009, **131**, 3826–3827; C. Paris, F. Geinguenaud, C. Gouyette, J. Liquier and J. Lacoste, *Biophys. J.*, 2007, **92**, 2498–2506.

