Attenuation of guanine oxidation via DNA-mediated electron transfer in a crowded environment using small cosolutes

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Attenuation of guanine oxidation via DNA-mediated electron transfer in crowded environment using small cosolutes

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ABSTRACT:

Guanine oxidation induced by photoirradiation on a pyrene-modified oligonucleotide was investigated under molecular crowding using small cosolutes such as glycerol. The efficiency of guanine photooxidation was suppressed in accordance with the increase in the concentration of glycerol. The results of photooxidation experiments using fully matched and mismatched DNA showed that guanine decomposition was mainly caused by DNA-mediated electron transfer (ET) in glycerol mixed solutions, as well as in diluted aqueous buffer solutions. Multiple factors can contribute to the suppression of guanine oxidation in crowded environments. However, our experimental results indicated that the attenuation of the DNA-mediated ET process suppressed guanine oxidation. On the other hand, experiments using ethylene glycol showed that guanine decomposition efficiency varies depending on the surrounding solvent. These results suggested that changes in the characteristics of the surrounding medium affect the DNA fluctuation, dominating DNA-mediated ET.

Introduction

Molecular crowding is a chief feature of the environment inside of cells, where a large number of molecules coexist at high density. It has been reported that a living cell has a concentration of up to 400 g/L of various biomolecules (40% occupancy).[1,2] Chemical reactions in cells that maintain life under such crowded conditions behave differently than those in the dilute solutions commonly used in the laboratory.[3-5] Crowded environments have different characteristics in terms of water activity, viscosity, and dielectric constants than dilute solutions, and these cause changes in the structure and stability of DNA, as well as in protein structures and enzymatic reactivities.[6]

Double-stranded DNA (dsDNA) consisting of four nucleobases carries all of the genetic information of life. On the other hand, dsDNA is considered to provide an ideal medium for electron transfer (ET) through π-stacking. In the 1990s, it was first reported that an electron hole injected by a photooxidant traveled long distances through stacking DNA bases.[7] Various chemical modifications of oligonucleotides have already been developed for detailed investigation of the DNA-mediated ET process through nucleobases containing electron donors and acceptors at given distances.[8-12]

Among the natural DNA nucleobases, guanine acts as an electron hole acceptor. A hole injected from a photooxidant migrates through π-stacking DNA bases and finally causes oxidative damage at the guanine base, particularly at the 5'-G of a continuous guanine tract.[13,14] Oxidized guanine bases can be regarded as a potent mutagenic lesions in the genome.[15,16] Currently, a great deal of attention is being focused on the in vivo function of ET within DNA.[17,18] It was recently reported that ET through DNA may be involved in the action of DNA primase.[19] The behavior of DNA-mediated ET in vivo is also expected to be different from that in dilute aqueous solution, but no studies have thus far been conducted on ET in DNA in a crowded molecular medium.

Very recently, we first reported the efficiency of guanine oxidation induced by a photooxidant in fully matched and mismatched DNA under crowded conditions.[20] However, the amount of guanine photooxidation in a crowded environment can be affected not only by the efficiency of DNA-mediated ET, but also by multiple factors depending on environmental changes. The mechanism by which crowding influences DNA-mediated ET has yet to be clarified. In order to obtain mechanistic information, we here investigated guanine photooxidation via DNA-mediated ET under crowded environments with newly designed oligonucleotides based on those used in our previous

report. That is, 5-(pyrenylethynyl)-2'-deoxyuridine (^{Py}U) was used as a hole injector in DNA, and a guanine tract in the same DNA assembly was used as a marker of photooxidation induced by hole trapping.[20] Then, the distance between the ^{Py}U and continuous G tract was adjusted; DNAs used in the present study had five adenines embedded between ^{Py}U and GG, while those used in our previous study had three.

To prepare a crowded environment, glycerol was used as a cosolute at high concentration in buffer solutions. The results showed that the addition of glycerol inhibited guanine decomposition induced by photoirradiation of the pyrene moiety in dsDNA. Photoirradiation experiments using oligonucleotides containing no inserted base pairs between the ^{Py}U and continuous G tract provided information on the DNA-mediated ET process attenuated by the addition of glycerol. Added ethylene glycol also attenuated the efficiency of guanine decomposition, however, the behavior of decomposition in ethylene glycol solution was slightly different from that in glycerol solution. These results suggest that changes in the local environment around DNA were induced by crowded conditions and affected the formation of ET-active conformation of DNA, as we discuss below.

Results and discussion

Design and properties of pyrene-modified oligonucleotides

Fig. 1 presents the sequences of pyrene-modified oligonucleotides used in this study and the structures of ^{Py}U. A pyrene molecule linked to the 5-position of uracil was placed in a major groove of dsDNA, thus ensuring retention of the Watson-Crick base pairing with adenine.[21] The ^{Py}UA$_5$GG sequence is preferable for investigating DNA-mediated ET under crowded conditions without the effect of through-space ET resulting from the disturbance of the local structure around ^{Py}U and a mismatched base pair.

In DNA I (^{Py}UA$_5$GG/T), a hole injected by the irradiation of ^{Py}U passes through a continuous sequence of five AT base pairs and is finally trapped at the GG tract with the lowest oxidation potential. The generated guanine radical cation ($G^{\bullet+}$) at the guanine continuous tract reacts with water or oxygen to give several oxidized bases such as 8-oxo-7,8-dihydro-2'-deoxyguanosine (8oxoG), 2,5-diamino-4H-imidazol-4-one (Iz), etc.[22-24] Other guanine sites for pairs with cytosines in dsDNA were replaced by inactive inosines (inosine, I). DNA II (^{Py}UA$_5$GG/A) and III (^{Py}UA$_5$GG/C) were designed to examine the effects of inserted mismatched base pairs, A/A and A/C, between the ^{Py}U and

continuous G tract. DNA IV (^{Py}UGG), in which the ^{Py}U and GG are adjacent, was designed to compare guanine decomposition efficiency in the absence of the DNA-mediated ET process under various conditions.

Aliquots of single-stranded (ss) DNA V (ss^{Py}UA$_5$GG) and VI (ss^{Py}UGG) were prepared for comparison with the properties of dsDNA. The representative UV-visible absorption spectra of dsDNA under various glycerol concentrations are shown in Fig. S1 (see Supporting Information). The absorption band of the conjugated pyrene moiety in the range from 340–420 nm allows for selective irradiation without excitation of DNA. Table 1 contains the T_m values for dsDNA I–IV under various cosolute concentrations. Consistent with a previous report for unmodified DNA,[25] the CD spectra indicated that the B-form conformations of the duplexes were unchanged by the addition of glycerol (Fig. 2). Thus, it was confirmed that the overall double helical structures of all DNA in glycerol mixed solutions were maintained at room temperature.

Photooxidation of pyrene-modified DNA in glycerol mixed solutions

Upon selective irradiation of the pyrene moiety (λ_{ex} > 350 nm) in DNA, guanine bases were oxidized and decomposed. The disappearance of deoxyguanosine (dG) was analyzed by HPLC after centrifugal filtration for purification and enzymatic digestion of each irradiated DNA sample solution. The HPLC profiles showed no obvious peak of decomposed bases via G$^{\bullet+}$, presumably due to the low yield (Fig. 3). It has been reported that photooxidized products of guanine are produced depending on various DNA conditions.[24] The amounts of other decomposed bases were almost negligible compared to those of dG.

Selective irradiation of ^{Py}U for 10 min in fully matched dsDNA I in aqueous buffer solution led to a 24% decomposition of dG. On the other hand, dG decomposition of DNA I was decreased to 16%, 14%, 12%, and 11% in 10, 20, 30 and 40 wt % glycerol, respectively (Fig. 4a). A/A and A/C mismatched dsDNA in aqueous buffer solution for 10-min irradiation resulted in 16% and 10% of dG decomposition, respectively (Fig. 4c, 4d). It is known that the insertion of a mismatched base generally suppresses DNA-mediated ET, because ET through DNA reflects sequence integrity.[26] By adding glycerol, the guanine decompositions of both mismatched DNA were attenuated. The dG decompositions of A/A mismatched DNA II in 10, 20, 30, and 40 wt % glycerol, were 11%, 9.1%, 8.9%, and 7.5%, respectively. Meanwhile, dG decompositions of A/C

mismatched dsDNA III in 10, 20, 30, and 40 wt % glycerol, were 7.8%, 7.0%, 6.7%, and 7.0%, respectively.

On the other hand, the amount of dG decomposition of DNA V (ss^{Py}UA$_5$GG) increased with increasing glycerol concentration (Fig. 4b). DNA V in aqueous buffer solution showed 16% dG decomposition for 10-min irradiation. The dG decompositions of DNA V in 10, 20, 30, and 40 wt % glycerol, were 18%, 23%, 26%, and 37%, respectively. The dG decomposition in ssDNA V should be induced by through-space ET, because efficient DNA-mediated ET occurs only between well-stacked bases within B-form dsDNA.[20] These results suggest that guanine oxidation in dsDNA I-III was mainly caused by DNA-mediated ET, even in crowded solutions.[27]

Changing the characteristics of the surrounding environment by adding glycerol affects all stages of the guanine photooxidation process (hole injection, DNA-mediated ET, and hole trapping). For example, the addition of glycerol changes the optical properties of ^{Py}U, the ET rate constant through DNA π-stacking, and the reactivity of $G^{\bullet+}$ with water or oxygen. It is also the possible that added glycerol might influence the guanine decomposition efficiency as a radical scavenger. The amount of dG decomposition of DNA I-III reflects all these changes. Therefore, dsDNA IV (^{Py}UGG) was designed to evaluate dG decomposition efficiency without the stage of DNA-mediated ET.[28]

Upon selective photoirradiation (λ_{ex} > 350 nm) of DNA IV, efficient dG decomposition (21%) was observed with shorter irradiation time (1 min), and a slight increase in the amount of dG decomposition was found with an increase in glycerol concentration (Fig. 5a). DNA VI (ss^{Py}UGG) in aqueous buffer solution showed 6.3% of dG decomposition with 1-min irradiation (Fig. 5b). The amount of dG decomposition in ssDNA VI was slightly increased by glycerol, however, the efficiency was considerably less than that of dsDNA IV. The dG decomposition in ssDNA VI should be induced by through-space ET between adjacent ^{Py}U and GG. From the results presented in Figs. 4a and 5a, we found that the suppression of guanine decomposition within dsDNA I (Fig. 4a) was mainly caused by the suppression of the ET process through DNA.

We measured that fluorescence spectra of dsDNA I and IV and their corresponding ssDNA V and VI with various the glycerol concentrations (Fig. 6). The florescence of ^{Py}U in ssDNA (Fig. 6c, d) was quenched more than that of ^{Py}U in dsDNA (Fig. 6a, b). These results were consistent with the occurrence of through-space ET between the excited ^{Py}U and the GG site in ssDNA. As the glycerol concentration increased, the

fluorescence intensity of ^{Py}U showed a tendency to increase. This result seemed reasonable, because a higher concentration of glycerol causes a decrease in the dielectric constant of the solvent. Our results also showed that the addition of glycerol influenced the optical properties of ^{Py}U. Moreover, the fluorescence maxima (λ_{max}) of DNA in high-concentration glycerol solutions were slightly blueshifted compared to those in diluted aqueous buffer solutions, suggesting that the local environment changed around ^{Py}U depending on the glycerol solutions.

In dsDNA IV, the distance between ^{Py}U and the GG tract is small. However, since the fluorescence intensity of dsDNA IV was only slightly lower than the fluorescence intensity of dsDNA I, the contribution of through-space ET in dsDNA IV was estimated to be small. It is considered that the florescence quenching of the pyrene moiety in modified dsDNA was mainly caused by the hole injection into a conjugated uracil moiety, which resulted in ET through π-stacked nucleobases. Despite the fact that the fluorescence quenching was larger in ssDNA than in dsDNA, the amount of decomposed guanine in ssDNA was much smaller. It is known that the rapid back ET between an adjacent electron acceptor and donor suppresses net DNA oxidation.[29,30] Thus, even if some through-space ET occurred in dsDNA IV, the amount of guanine degradation induced by through-space ET was considered to be small.

Photooxidation of pyrene-modified DNA in ethylene glycol mixed solutions

In order to investigate the environmental characteristics that affect DNA-mediated ET, another set of photooxidation experiments for pyrene-modified dsDNA I and IV were conducted in a crowded environment containing ethylene glycol. The CD spectra measurement indicated that, as with glycerol, the overall conformations of the duplexes were unchanged by the addition of ethylene glycol (Fig. 7). Upon photoirradiation (λ_{ex} > 350 nm, 10 min), dG decomposition was observed in dsDNA I (^{Py}UA$_5$GG) in the presence of ethylene glycol as in glycerol (Fig. 8a). The dG decomposition of dsDNA I in ethylene glycol mixed solutions also decreased with increasing concentrations of ethylene glycol, and was almost the same or slightly higher than in glycerol mixed solutions.

On the other hand, unlike in the glycerol mixed solvent, the amount of guanine decomposition of dsDNA IV (^{Py}UGG) decreased as ethylene glycol concentration increased (Fig. 8b). Similar dielectric constant values have been reported for glycerol

and ethylene glycol.[31,32] The measured viscosities of 10 wt % glycerol and ethylene glycol solutions showed the same values, while the values of 20–40 wt % ethylene glycol solutions were slightly lower than those of glycerol (Table S1). Although the bulk properties of glycerol mixed solution and ethylene glycol mixed solution appeared similar, the experimental results suggested that the environmental characteristics in the hydration zone around DNA were different.

Crowding effects on DNA-mediated ET

Because of the hydration of the phosphate group and base pair, the water density around DNA is increased over that of bulk water,[33] and theoretical investigation has shown that water molecules are strongly packed at high density in the minor grooves of B-form DNA.[34] The lower mobility of water molecules bound to DNA makes the dielectric constant around DNA considerably lower than that of bulk water.[35] The dielectric constant of the cosolute mixed buffer solution around DNA is also expected to be much lower than that in bulk water.

It has been reported that ethylene glycol replaces water in the first hydration shell around B-form DNA.[36] Like ethylene glycol, it is expected that added glycerol will be replaced by water, but the orientation of water molecules and cosolute molecules around DNA in glycerol mixed solvent should be different from those in ethylene glycol mixed solvent because of the difference in structure and number of hydroxyl groups contained in each cosolute. It is also expected that depending on the cosolute around DNA, the orientation of water molecules would affect the reaction between $G^{\bullet+}$ and water. The idea that there are differences in the interactions of these cosolutes with DNA is consistent with the result that the melting temperatures differed depending on the type of cosolute (Table 1).[37]

On the other hand, a low dielectric constant in the surrounding medium typically delocalizes a hole in DNA, and decreases the solvent reorganization energy, λ_s, in the ET process.[35] Thus, a lower dielectric constant around an electron donor and acceptor is generally advantageous for efficient ET. Our results, however, showed an attenuation of DNA-mediated ET in a low-dielectric crowded medium. We expected that the addition of cosolutes would affect the formation of the ET-active conformation of DNA for gated ET.[38-41] Local DNA conformational change due to thermal fluctuations has been reported to alter the rate of the ET process through DNA.[42] It has also been

reported that DNA-mediated ET is influenced by the fluctuations of the counterions and water molecules around DNA.[43]

Hydroxyl groups of glycerol and ethylene glycol can form hydrogen bonds with the atoms in bases and backbones in DNA,[36] and the network and strength of hydrogen bonds around DNA should vary depending on the cosolutes. It is considered that such hydrogen bonding around DNA in crowded environments changes the thermal fluctuation for gated ET through well-coupled alignment of the bases. Although the influence on the structure of the entire DNA by environmental change seemed subtle in the measured CD spectra, our results suggested that the variations in structural fluctuations of DNA in a crowded environment affected DNA-mediated ET and DNA oxidation.

To investigate further, photooxidation experiments were also performed in acetonitrile mixed solutions for pyrene-modified dsDNA I and IV. Acetonitrile acts as a dehydrating and non-hydrogen-bonding agent.[44] By the addition of acetonitrile, dG decomposition in DNA I was attenuated a little; however, decomposition was more strongly suppressed in DNA IV (Fig. S2). The suppression of decomposition in DNA IV may derive from the dehydration around DNA, because of the irreversible oxidation of DNA caused by the reaction between $G^{\bullet+}$ and water or oxygen, as mentioned above. Alternatively, although the CD spectra indicated that the overall structure of B-form DNA was unchanged by the addition of acetonitrile (Fig. S3), as the acetonitrile greatly destabilized dsDNA (Table S2), it is possible that the local structure around ^{Py}U and GG was changed.[20] Thus, it was difficult to accurately verify the efficiency using this system, although DNA-mediated ET might be promoted in acetonitrile mixed solutions.

Conclusions

We found that the guanine photooxidation induced by DNA-mediated ET in a pyrene-modified oligonucleotide was attenuated under a crowded environment containing small cosolutes. Experiments using oligonucleotides adjacent to the pyrene moiety and guanine continuous tract showed that the suppression of ET through base stacking occurred under crowding using glycerol. It is expected that these environments created by small cosolutes change the hydration properties around DNA, and affect the electronic coupling between base pairs by structural fluctuation, which dominated DNA-mediated ET. In the intracellular environment, various properties of the local environment change continuously, according to the situation. The cellular functions, including DNA-

mediated ET in vivo, would vary according to changes in the local environment containing enormous biomolecules.

Experimental section

Materials

HPLC-grade DNAs were purchased from Japan Bio Services Co., LTD. Crotalus adamanteus venom phosphodiesterase I (Worthington), and Alkaline Phosphatase, Calf Intestinal (Promega) were used for the enzymatic digestion of oligonucleotides. Glycerol was purchased from Nacalai Tesque, Japan. Ethylene glycol was purchased from Wako Pure Chemical Industries, Ltd. Aliquots of dsDNA I–IV samples were prepared by annealing equimolar amounts of desired DNA complements. The samples were heated at 85 °C for 3 min, then cooled slowly. Before each experiment, a concentrated aqueous buffer solution of cosolute was added to a DNA sample solution. All aqueous solutions utilized ultra pure water (Komatsu Electronics, UL-pure).

UV-vis, CD and fluorescence spectral measurements

Absorption spectra were obtained using a JASCO V-730BIO spectrophotometer at room temperature with a 1-mm path length cell. Fluorescence spectra were recorded on a JASCO spectrofluorophotometer (FP-6300) using a one-drop measurement unit and a UV cut-off filter $\lambda > 370$ nm for scattering. The excitation wavelength was 340 nm. CD measurements were carried out on a JASCO J-720W spectropolarimeter (Japan Spectroscopic Co., Ltd.) using a one-drop measurement unit (1-mm path length) for dsDNA in glycerol and ethylene glycol mixed solutions, and using a micro cell holder with MAC-10 cell (10-mm path length) for dsDNA in acetonitrile mixed solutions.

Melting temperature measurements

Melting temperatures (T_m) of all the duplexes were measured using a JASCO V-730BIO spectrophotometer with a temperature control attachment. Absorption at 260 nm (A260) of equimolar DNA complements (4.0 μM in 100 mM NaCl, 50 mM Tris-HCl buffer pH7.4) were measured every 0.5 ºC/min from 20–80 °C for glycerol and ethylene glycol mixed solutions, and from 20–70 °C for acetonitrile mixed solutions.

Photooxidation experiments

Aliquots (4.0 μM DNA, 100 mM NaCl, 50 mM Tris-HCl, pH 7.4, total volume 30 μL) were prepared for irradiation. DNA solutions were irradiated with a Xe lamp (300 W; Asahi Spectra Co. Ltd.; MAX-303) through a UV cut-off filter (LUX350). The irradiated solutions were filtered using an Amicon membrane NMWL of 3 kDa (Merck) to remove small cosolutes. Filtrated DNA samples were digested by incubation with both alkaline phosphatase and phosphodiesterase I at 37 ºC overnight in order to yield the free nucleosides, and digested samples were analyzed by reversed phase HPLC monitored at 254 nm. HPLC analyses were performed with a JASCO Chromatograph, EXTREMA using a CHEMCOBOND 5-ODS-H column (4.6x150 mm) eluted with 0.05 M ammonium acetate buffer containing acetonitrile (gradient: 3–9 % over 25 min). The percentage decomposition of guanine was determined using dC as an internal standard for all HPLC traces. Irradiation experiments were repeated at least three times to give average results.

Measurements of viscosity

Viscosities (η) of aqueous glycerol and ethylene glycol solutions were measured using a Vibro viscometer SV-10 (A&D).

Electronic supplementary information (ESI) available:

UV-vis spectra of pyrene-modified oligonucleotides, and viscosities of aqueous glycerol and ethylene glycol solutions. The dG decomposition percentage, CD spectra, and melting temperatures of acetonitirile mixed solutions. See DOI:

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Conflicts of interest

There are no conflicts to declare.

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DNA I : $^{PY}UA_5GG/T$

```
5'-TTT CAA TTT ATT CAC TAA XAA AAA GGA ACA TCT TTC TTA ATA -3'
3'-AAA ITT AAA TAA ITI ATT ATT TTT CCT TIT AIA AAI AAT TAT -5'
```

DNA II : $^{PY}UA_5GG/A$

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5'-TTT CAA TTT ATT CAC TAA XAA AAA GGA ACA TCT TTC TTA ATA -3'
3'-AAA ITT AAA TAA ITI ATT ATT ATT CCT TIT AIA AAI AAT TAT -5'
```

DNA III : $^{PY}UA_5GG/C$

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5'-TTT CAA TTT ATT CAC TAA XAA AAA GGA ACA TCT TTC TTA ATA -3'
3'-AAA ITT AAA TAA ITI ATT ATT CTT CCT TIT AIA AAI AAT TAT -5'
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DNA IV : ^{PY}UGG

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5'-TTT CAA TTT ATT CAC TAA XGG AAA AAA ACA TCT TTC TTA ATA -3'
3'-AAA ITT AAA TAA ITI ATT ACC TTT TTT TIT AIA AAI AAT TAT -5'
```

DNA V : $ss^{PY}UA_5GG$

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5'-TTT CAA TTT ATT CAC TAA XAA AAA GGA ACA TCT TTC TTA ATA -3'
```

DNA VI : $ss^{PY}UGG$

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5'-TTT CAA TTT ATT CAC TAA XGG AAA AAA ACA TCT TTC TTA ATA -3'
```

X = ^{PY}U

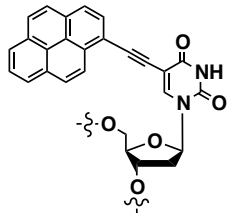

Fig. 1 The sequences of DNA I-VI with a ^{Py}U structure.

Table 1. Melting temperatures (T_m) for dsDNA I–IV in the absence and presence of cosolutes.

Cosolute	T_m, °C	T_m, °C	T_m, °C	T_m, °C	Cosolute	T_m, °C
	DNA I	DNA II	DNA III	DNA IV		DNA I
none	54.0	50.8	49.8	54.9	none	53.7
10 wt % glycerol	52.0	48.9	47.9	53.0	10 wt % ethylene glycol	51.5
20 wt % glycerol	50.1	47.0	46.0	51.4	20 wt % ethylene glycol	48.6
30 wt % glycerol	48.1	45.1	44.2	49.3	30 wt % ethylene glycol	45.3
40 wt % glycerol	46.1	43.0	42.3	47.4	40 wt % ethylene glycol	42.5

Experimental conditions: [DNA duplex] = 4.0 μM in 50 mM Tris-HCl, pH7.4, 100 mM NaCl. T_m is determined by monitoring the UV absorption at 260 nm. The error of T_m was less than 0.5 °C.

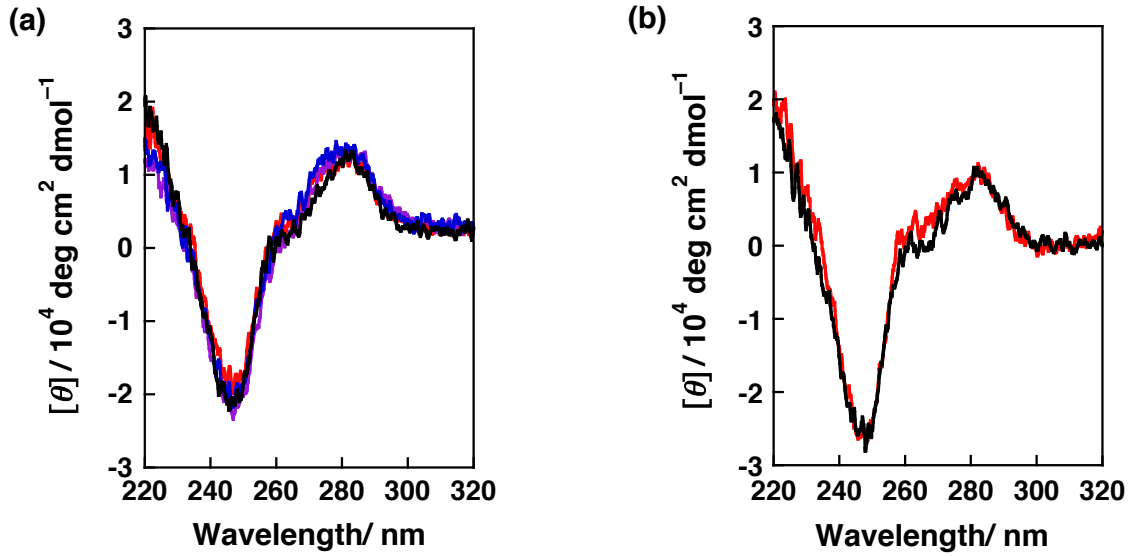

Fig. 2 (a) CD spectra of DNA I in aqueous buffer solution (black line), DNA I (red line), DNA II (purple line), and DNA III (blue line) in 40 wt % glycerol solution. (b) CD spectra of DNA IV in aqueous buffer solution (black line) and in 40 wt % glycerol solution (red line). These spectra were obtained using a one-drop measurement unit. Experimental conditions: [DNA duplex] = 4.0 μM, and [NaCl] = 100 mM in pH 7.4 Tris-HCl buffer (50 mM).

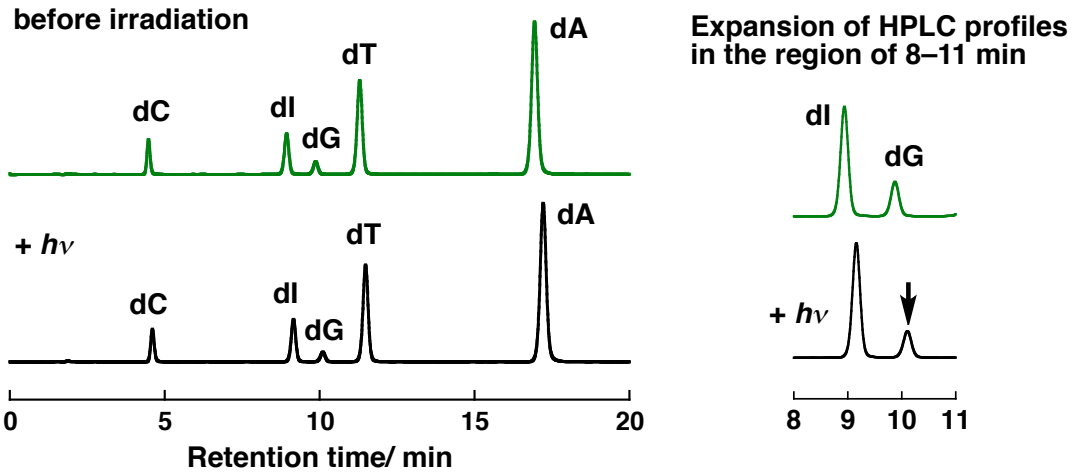

Fig. 3 Representative HPLC trace monitoring the photoreaction of DNA I in aqueous buffer solution at 254 nm obtained before and after 10-min irradiation (λ_{ex} > 350 nm), purification, and subsequent digestion of aliquots.

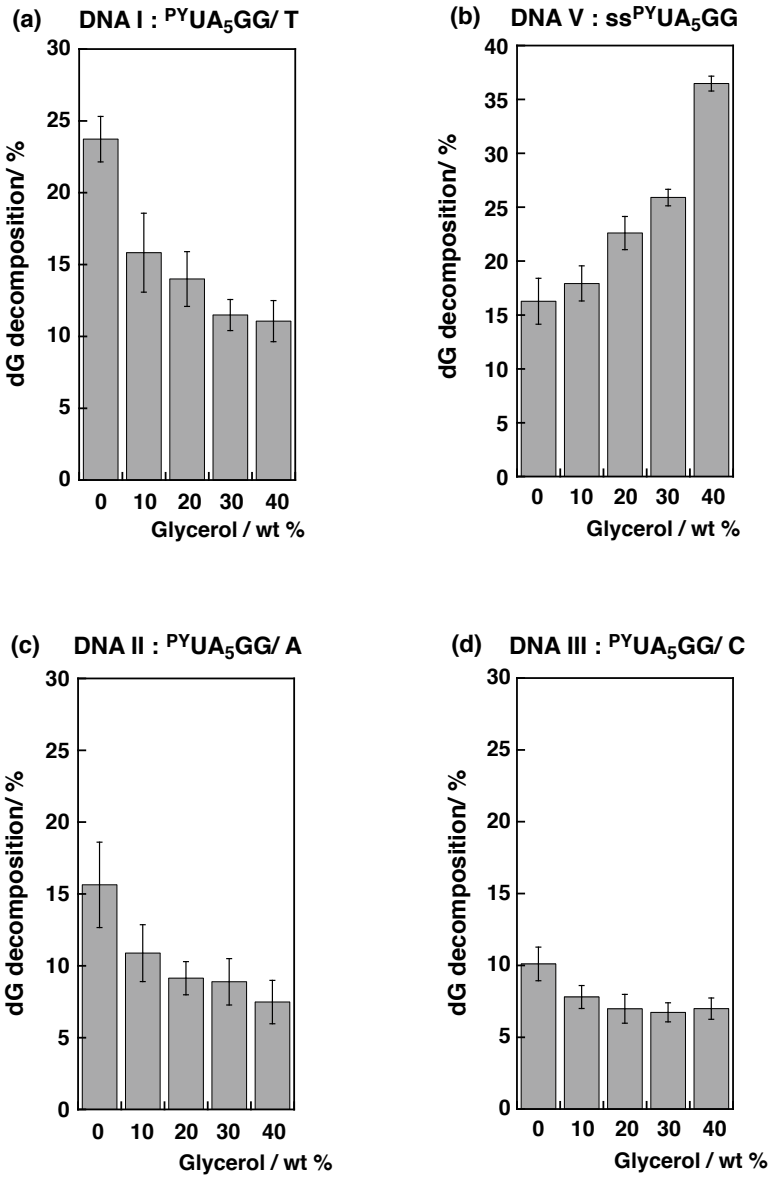

Fig. 4 dG decomposition percentages of the pyrene-modified oligonucleotides (a) DNA I, (b) DNA V (single strand), (c) DNA II, and (d) DNA III obtained from photoirradiation (λ_{ex} > 350 nm, 10 min) in an aqueous buffer solution, and 10 – 40 wt % glycerol. The conditions were as follows: [DNA] = 4.0 μM in pH 7.4 Tris-HCl buffer (50 mM), and [NaCl] = 100 mM.

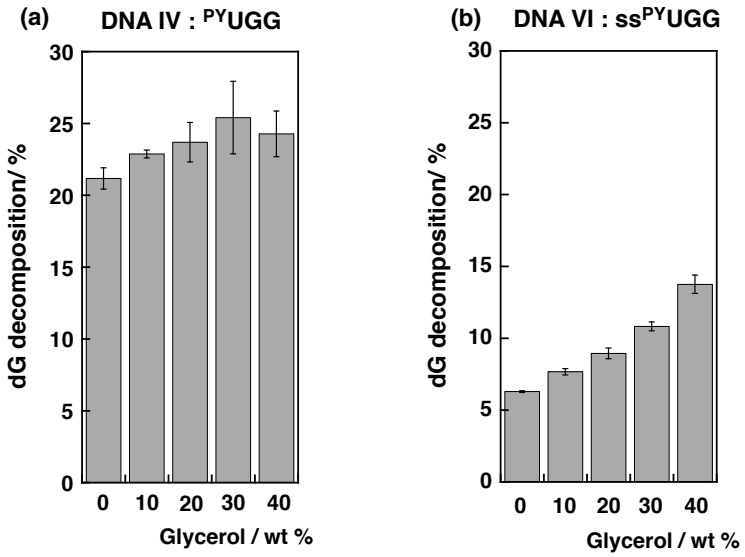

Fig. 5 dG decomposition percentages of the pyrene-modified oligonucleotides (a) DNA IV, and (b) DNA VI (single strand) obtained from photoirradiation (λ_{ex} > 350 nm, 1min) in an aqueous buffer solution, and 10 – 40 wt % glycerol. The conditions were as follows: [DNA] = 4.0 μM in pH 7.4 Tris-HCl buffer (50 mM), and [NaCl] = 100 mM.

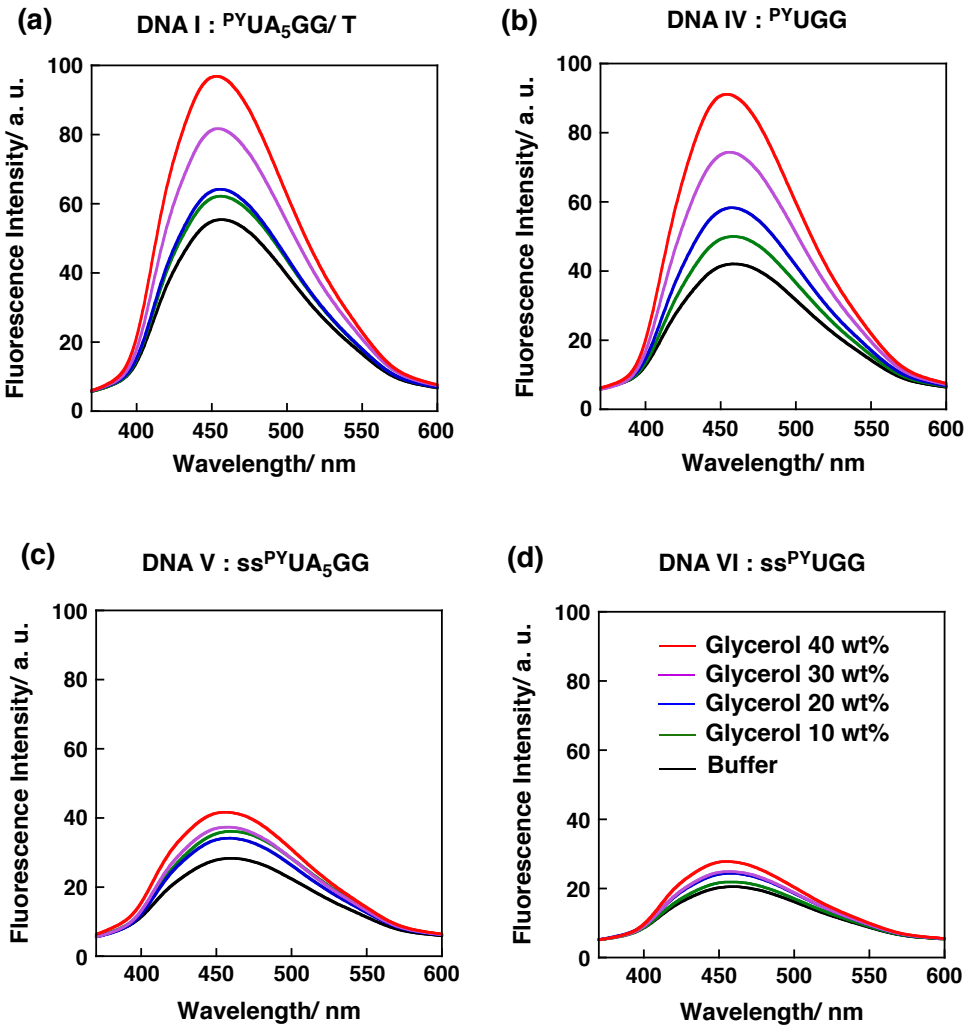

Fig. 6 Fluorescence spectra of (a) DNA I, (b) DNA IV, (c) DNA V, and (d) DNA VI in aqueous buffer solutions (black line), 10 wt % (green line), 20 wt % (blue line), 30 wt % (purple line), and 40 wt % (red line) glycerol solutions. The excitation wavelength is 340 nm. These spectra were obtained using a one-drop measurement unit. The experimental conditions were as follows: [DNA duplex] = 4.0 μM, and [NaCl] = 100 mM in pH 7.4 Tris-HCl buffer (50 mM).

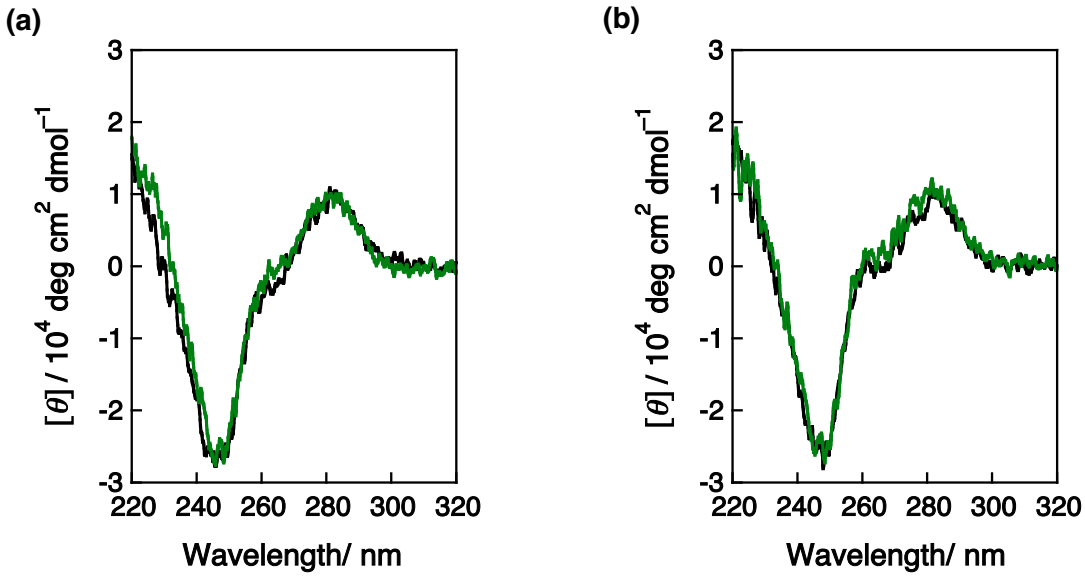

Fig. 7 (a) CD spectra of DNA I in aqueous buffer solution (black line) and in 40 wt % ethylene glycol solution (green line). (b) CD spectra of DNA IV in aqueous buffer solution (black line) and in 40 wt % ethylene glycol solution (green line). These spectra were obtained using a one-drop measurement unit. Experimental conditions: [DNA duplex] = 4.0 μM, and [NaCl] = 100 mM in pH 7.4 Tris-HCl buffer (50 mM).

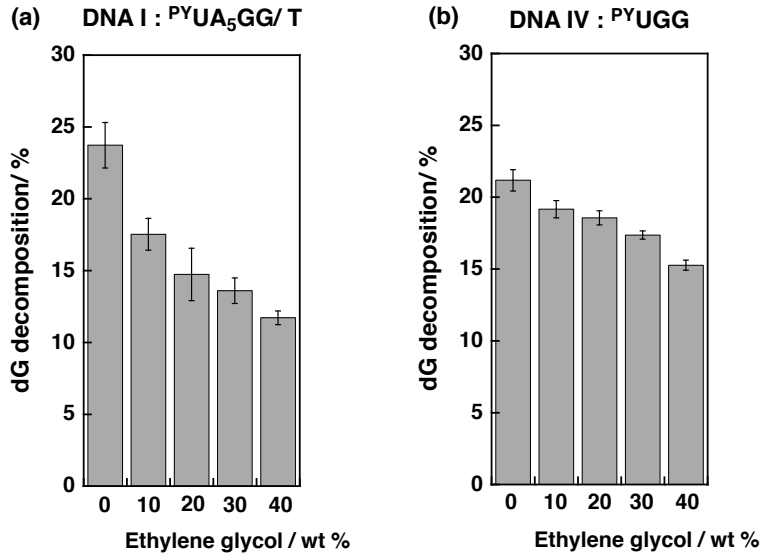

Fig. 8 dG decomposition percentages of pyrene-modified oligonucleotides (a) DNA I from photoirradiation (λ_{ex} > 350 nm, 10 min), and (b) DNA IV obtained from photoirradiation (λ_{ex} > 350 nm, 1 min) in an aqueous buffer solution, and 10 – 40 wt % ethylene glycol. The conditions were as follows: [DNA] = 4.0 μM in pH 7.4 Tris-HCl buffer (50 mM), and [NaCl] = 100 mM.