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Spectroscopic Investigation of Na^+-Dependent Conformational Changes of a Cyclobutane Pyrimidine Dimer-Repairing Deoxyribozyme

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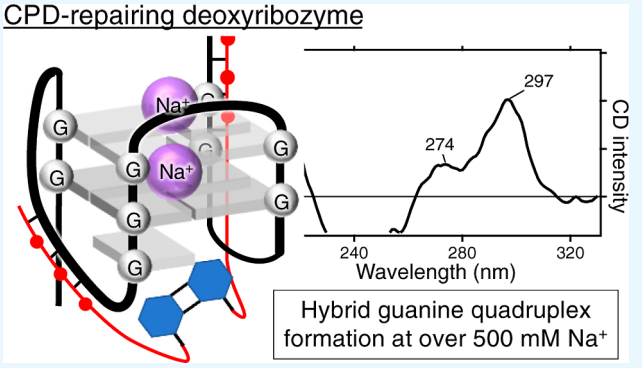
ABSTRACT: UV1C is an enzymatically active DNA sequence (deoxyribozyme, DNAzyme) that functions as a cyclobutane pyrimidine dimer (CPD) photolyase. UV1C forms parallel guanine quadruplexes (G-quadruplexes) with a DNA substrate in the presence of 240 mM Na^+, the structure of which is important for the enzymatic activity. To investigate the repair mechanism of CPD by UV1C, we designed light-induced Fourier transform infrared (FTIR) spectroscopy. Prior to FTIR measurements, circular dichroism (CD) spectroscopy was conducted to determine the Na^+ concentration at which the most G-quadruplexes were formed. We found that UV1C also forms a hybrid G-quadruplex structure at over 500 mM Na^+. By assuming a concentration equilibrium between G-quadruplexes and Na^+, 1.3 and 1.8 Na^+ were found to bind to parallel and hybrid G-quadruplexes, respectively. The hybrid G-quadruplex form of UV1C was also suggested to exhibit photolyase activity. Light-induced FTIR spectra recorded upon the photorepair of CPD by UV1C were compared for parallel G-quadruplex-rich and hybrid G-quadruplex-rich samples. Spectral variations were indicative of structural differences in parallel and hybrid G-quadruplexes before and after CPD cleavage. Differences were also observed when compared to the CPD repair spectrum by CPD photolyase. The spectral differences during CPD repair by either protein or DNAzyme suggest the local environment of the substrates, the surrounding protein, or the aqueous solution.



1. INTRODUCTION

Living organisms are prone to DNA damage, and they also possess mechanisms to repair this.[1−3] Among chemical species of damaged DNA, ultraviolet (UV)-induced damaged DNA includes cyclobutene pyrimidine dimers (CPDs) and pyrimidine-pyrimidone (6-4) photoproducts ((6-4)PPs), which are formed with covalent bonds between adjacent pyrimidine bases.[4,5] DNA photolyases are enzymes that directly repair UV-damaged DNA into normal bases using near-UV/blue light energy. CPD photolyase and (6-4) photolyase are photolyases that correspond to the structure of UV-damaged DNAs.

Both CPDs and (6-4)PPs are formed with covalent bonds between neighboring pyrimidine bases under UV light (~260 nm) irradiation. For the CPD formation, a [2 + 2] cycloaddition reaction occurs at C5═C6 of adjacent thymines, forming a four-membered ring, while (6-4)PP is formed with covalent bonds at C6 of 5′ thymine and C4═O of 3′ thymine.[4,5]

DNA photolyase binds flavin adenine dinucleotide (FAD), which acts as an electron donor to DNA lesions upon photoillumination. In photolyases, the reduced form of FAD is responsible for the repair.[4] On the other hand, CPD can be cleaved without photolyases by receiving an electron from a neighboring guanine base through photooxidation.[6,7] CPDs can be cleaved when an appropriate electron donation occurs in an enzymatic or a nonenzymatic reaction.

In 2004, a 42-mer DNA sequence, UV1C, was reported, which has CPD photolyase activity.[8] DNAs that possess enzymatic activities are called deoxyribozymes (DNAzymes), and the first DNAzyme reported in 1994 was a ribonuclease.[9] There are no known DNAzymes in vivo, and all DNAzymes reported were constructed (or found) by in vitro selection methods with appropriate selection processes.[10,11] UV1C was obtained through in vitro selection, which catalyzed >300 nm light-induced repair of DNA lesions called thymine dimer-containing primers (TDPs). Because UV1C is catalytically active using light >300 nm which normal DNA does not absorb and requires monovalent cations, it was predicted that DNA forms a special structure such as a guanine quadruplex

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Table 1. Oligonucleotide Names and Sequences

T1	5′-AGGATCTACATGTAT-3′
T2	5′-TGTGTGCGTACGAGTATATG-3′
splint	5′-CTCGTACGCACACAATACATGTAGA-3′
UV1C	5′-GGAGAACGCGAGGCAAGGCTGGGAGAAATGTGGATCACGATT-3′
LMP	5′-AGGATCTACATGTATTGTGTGCGTACGAGTATATG-3′

(G-quadruplex) structure.[8] Sen and co-workers later revealed that the UV1C forms parallel G-quadruplexes in complex with TDP by cross-linking experiments[12,13] and circular dichroism (CD) spectroscopy.[14] They also investigated the substrate specificity and that UV1C can repair CPD with a phosphodiester linkage in between [ligated dimer primer (LDP)].[15] Since photoequilibrium occurs between the CPD and the thymines in the complex between UV1C and LDP, it was revealed that both LDP and a repaired (undamaged) oligonucleotide [ligated monomeric primer (LMP)] remain bound to UV1C during photoreaction.

In this study, light-induced Fourier transform infrared (FTIR) spectroscopy was performed in UV1C with TDP to investigate the recognition and repair mechanisms, as we applied it to DNA photolyases.[16−20] Here, we chose TDP as the substrate instead of LDP because we expected to observe the repair and dissociation of TDP from UV1C by light-induced FTIR difference spectroscopy. Regarding the use of LDP as the substrate, we were concerned that if repair and redamage (reversion to the original state) occurred, and the FTIR difference spectra would not be satisfactorily observed.

To estimate the appropriate Na^+ concentration for FTIR measurements, CD spectroscopy was performed at various Na^+ concentrations. Interestingly, CD spectra showed Na^+ concentration dependence, suggesting the formation of a hybrid G-quadruplex at over 500 mM Na^+. Light-induced FTIR difference spectra were measured for TDP cleavage by UV1C formed at different Na^+ concentrations. The difference spectrum of TDP repair by a bacterial CPD photolyase was also measured. Based on the obtained infrared spectra, the structures and environments of TDP and UV1C were discussed.

2. MATERIALS AND METHODS

2.1. Construction of TDP Substrate. DNA oligonucleotides were prepared using corporate DNA synthesis services (Rikaken, Japan; Eurofins Genomics K.K., Japan; Table 1).

T1, T2, and a splint were each mixed at a final concentration of 0.4 mM in the presence of 40 mM $MgCl_2$. To anneal three oligonucleotides into double-stranded DNA, the mixture was set at 95 °C for 3 min and cooled to 30 °C for over 1.5 h (annealing). For TDP formation, 100 mM acetophenone in acetone was added to the annealed samples at a final concentration of 5 mM. Irradiation at 340 nm was supplied by a xenon lamp equipped with a 340 nm filter at a light intensity of about 240 mJ cm^{-1} min^{-1} (MAX-301 and LX0340 filter, Asahi Spectra, Japan). UV-irradiated oligonucleotides were subjected to urea polyacrylamide gel electrophoresis (urea-PAGE) (Figures S1 and S2). Among various lengths of DNA fragments, 35-mer-long DNA was supposed as TDP. By comparing the formation of 35-mer DNA of different irradiation times from 1 to 8 h, UV irradiation was done for 2 h for TDP formation (Figure S1).

Gel fragments containing 35-mer-long DNA were collected in 1.5 mL microtubes, and twice the amount of distilled water was added into the tubes. After the tubes were shaken at 37 °C overnight, gel debris was removed by filter devices (Ultrafree-MC, GV 0.22 μm, Merck, Germany). DNA was collected by alcohol precipitation and dissolved in distilled water. DNA concentrations were estimated by absorption at 260 nm. Purified DNA was confirmed by urea-PAGE (Figure S2).

2.2. Repair of TDP Substrate by UV1C under Light Illumination. The 35-mer-long DNA was mixed with UV1C and annealed at an appropriate Na^+ concentration. NaCl was used as the Na^+ source, and the annealed samples were illuminated with a 295−370 nm light source at a light intensity of about 600 mJ cm^{-1} min^{-1}. The illuminated samples were subjected to urea-PAGE (Figures S3 and S4). The 35-mer-long DNA was regarded as TDP because a DNA part was dissociated with 15 mer-long (T1) and 20 mer-long (T2) DNA fragments by illumination in the presence of both UV1C and Na^+ (Figure S3). Cleaved and remaining samples were estimated from the band intensity of gels using ImageJ2 software[21] (Figures S4 and 3). The band intensity data were obtained using a single electrophoresis result (Figure S4). In Figure 3, the means and standard deviations of the data were obtained using six electrophoresis results for 240 mM Na^+ and five electrophoresis results for 1500 mM Na^+. The data were analyzed by Igor Pro software (WaveMetrics, Inc., OR).

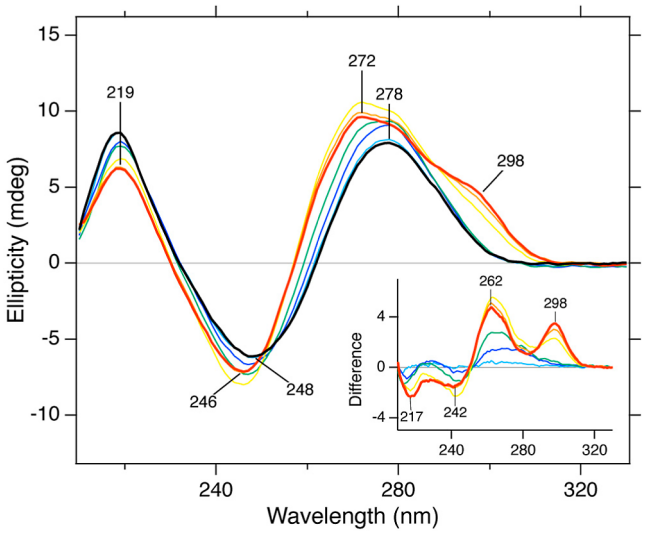

Figure 1. CD spectra of the 1 μM UV1C−LMP mixture in the absence/presence of NaCl. Black, 0 mM; light blue, 10 mM; blue, 50 mM; green, 100 mM; yellow, 500 mM; orange, 1000 mM; red, 1500 mM. The inset shows the difference spectra in the absence and presence of Na^+.

2.3. CD Spectroscopy. CD spectra were measured using a CD spectrophotometer equipped with a temperature controller set at 20 °C (J-1500 and PTC-510, JASCO, Japan). UV1C and LMP were each mixed at 1 μM in the presence of 0, 10, 50, 100, 500, 1000, or 1500 mM NaCl in 20 mM Tris−HCl (pH 8) buffer and annealed. For each annealed sample, 16 CD

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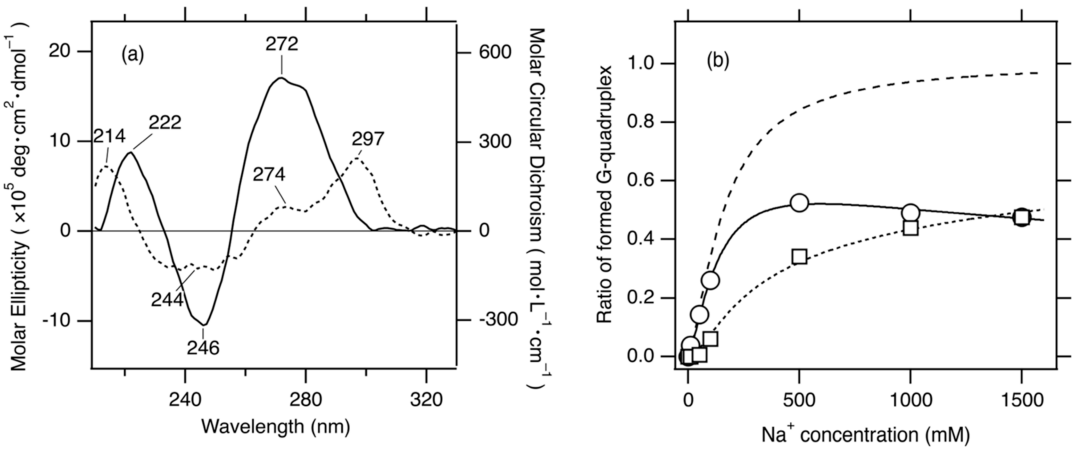

Figure 2. (a) Estimated CD spectra of the UV1C−TDP complex observed at lower (solid line) and higher (dotted line) Na^+ concentrations. The former suggests a parallel G-quadruplex, while the latter suggests a hybrid G-quadruplex. (b) The ratios of formed G-quadruplexes were plotted as a function of Na^+ concentration. Circles and squares represent rates of parallel and hybrid G-quadruplexes, respectively. Solid and dotted lines show the theoretical equations (Materials and Methods). Broken line shows the sum of formed G-quadruplex fractions. Fitting to the theoretical equations resulted in binding of 1.3 Na^+ for parallel G-quadruplexes and 1.8 Na^+ for hybrid G-quadruplexes.

measurements were averaged. Difference spectra in the presence or absence of appropriate Na^+ concentrations were calculated.

2.4. Spectral Decomposition. The Na^+-dependent CD spectra exhibited changes in the spectral intensity and spectral shapes. By assuming that CD spectral changes consist of two components, the difference spectra were calculated by the least-squares method using the solver addin in Excel software (Microsoft, WA). The relative ratios of the components present in the difference spectra at various Na^+ concentrations were calculated with the amount present in the spectra at 1500 mM Na^+ concentration as 1 (Figure S5). From the obtained difference spectra, the CD spectra derived from each G-quadruplex structure were calculated. The amplitude of the difference spectra when 100% of the original DNA was changed to the G-quadruplex was estimated and added to the CD spectrum measured without Na^+. To the extent that the sum of the ratios of both G-quadruplexes does not exceed 1, appropriate absorption coefficients in the range given in the literature were used to fit the equations below.[22]

2.5. Data Analysis of the Numbers of Na^+ and Equilibrium Constants for G-Quadruplex Formation. The numbers of Na^+ bound to UV1C−TDP complexes for G-quadruplex formation were estimated using the following equilibrium formulas

G-quadruplex at lower Na^+ concentrations

$$\mathrm{UV1C} + n_1\mathrm{Na}^+ \rightleftharpoons \mathrm{UV1C{\cdot}Na}^+_{n_1} \quad (1)$$

G-quadruplex at higher Na^+ concentrations

$$\mathrm{UV1C} + n_2\mathrm{Na}^+ \rightleftharpoons \mathrm{UV1C{\cdot}Na}^+_{n_2} \quad (2)$$

where n_1 and n_2 ($n_1 < n_2$) in formulae 1 and 2 are the numbers of bound Na^+ for the formation of G-quadruplexes at lower and higher Na^+ concentrations, respectively. The equilibrium constants for each reaction, K_1 and K_2, are given as follows

$$K_1 = \frac{[\mathrm{UV1C{\cdot}Na}^+_{n_1}]}{[\mathrm{UV1C}]_f[\mathrm{Na}^+]_f^{\,n_1}} \quad (3)$$

$$K_2 = \frac{[\mathrm{UV1C{\cdot}Na}^+_{n_2}]}{[\mathrm{UV1C}]_f[\mathrm{Na}^+]_f^{\,n_2}} \quad (4)$$

where $[\mathrm{UV1C{\cdot}Na}_{n_1}{}^+]$, $[\mathrm{UV1C{\cdot}Na}_{n_2}{}^+]$, $[\mathrm{UV1C}]_f$, and $[\mathrm{Na}^+]_f$ are concentrations of the G-quadruplex at lower Na^+ concentrations, G-quadruplex at higher Na^+ concentrations, free UV1C (UV1C that does not form G-quadruplexes), and Na^+ in solution (free Na^+), respectively. $[\mathrm{UV1C}]_I$ and $[\mathrm{Na}^+]_I$ are the UV1C and Na^+ concentrations initially added to the tubes, respectively. Therefore, the relationship among the concentration is expressed as follows

$$[\mathrm{UV1C}]_f + [\mathrm{UV1C{\cdot}Na}^+_{n_1}] + [\mathrm{UV1C{\cdot}Na}^+_{n_2}] = [\mathrm{UV1C}]_I \quad (5)$$

$$[\mathrm{Na}^+]_f + [\mathrm{UV1C{\cdot}Na}^+_{n_1}] + [\mathrm{UV1C{\cdot}Na}^+_{n_2}] = [\mathrm{Na}^+]_I \quad (6)$$

The $[\mathrm{UV1C}]_I$ in CD spectroscopy (1 μM) was much lower than $[\mathrm{Na}^+]_I$ (10−1500 mM); the concentration of $[\mathrm{Na}^+]_f$ hardly changed unless more than 1000 Na^+ were bound to one DNA molecule. Under the approximation of $[\mathrm{Na}^+]_f = [\mathrm{Na}^+]_I$ in eqs 3−6, the ratios of formed G-quadruplexes, $[\mathrm{UV1C{\cdot}Na}^+_{n1}]/[\mathrm{UV1C}]_I$ and $[\mathrm{UV1C{\cdot}Na}^+_{n2}]/[\mathrm{UV1C}]_I$, are represented as follows

$$\frac{[\mathrm{UV1C{\cdot}Na}^+_{n_1}]}{[\mathrm{UV1C}]_I} = \frac{K_1[\mathrm{Na}^+]_I^{n_1}}{1 + K_1[\mathrm{Na}^+]_I^{n_1} + K_2[\mathrm{Na}^+]_I^{n_2}} \quad (7)$$

$$\frac{[\mathrm{UV1C{\cdot}Na}^+_{n_2}]}{[\mathrm{UV1C}]_I} = \frac{K_2[\mathrm{Na}^+]_I^{n_2}}{1 + K_1[\mathrm{Na}^+]_I^{n_1} + K_2[\mathrm{Na}^+]_I^{n_2}} \quad (8)$$

The numbers of bound Na^+ (n_1 and n_2) and equilibrium constants (K_1 and K_2) were estimated by eqs 7 and 8 using Igor Pro software, respectively.

2.6. FTIR Spectroscopy. Almost equal amounts of UV1C and TDP were mixed and precipitated by 2-propanol. The precipitate was dissolved to a concentration of approximately 2−3 mM in either 240 mM NaCl and 20 mM Tris−HCl (pH 8) or 1500 mM NaCl and 20 mM Tris−HCl (pH 8). Then, 0.6 μL of the sample was put on a BaF_2 window and sandwiched with another window. Light-induced FTIR difference spectra upon TDP repair were measured according

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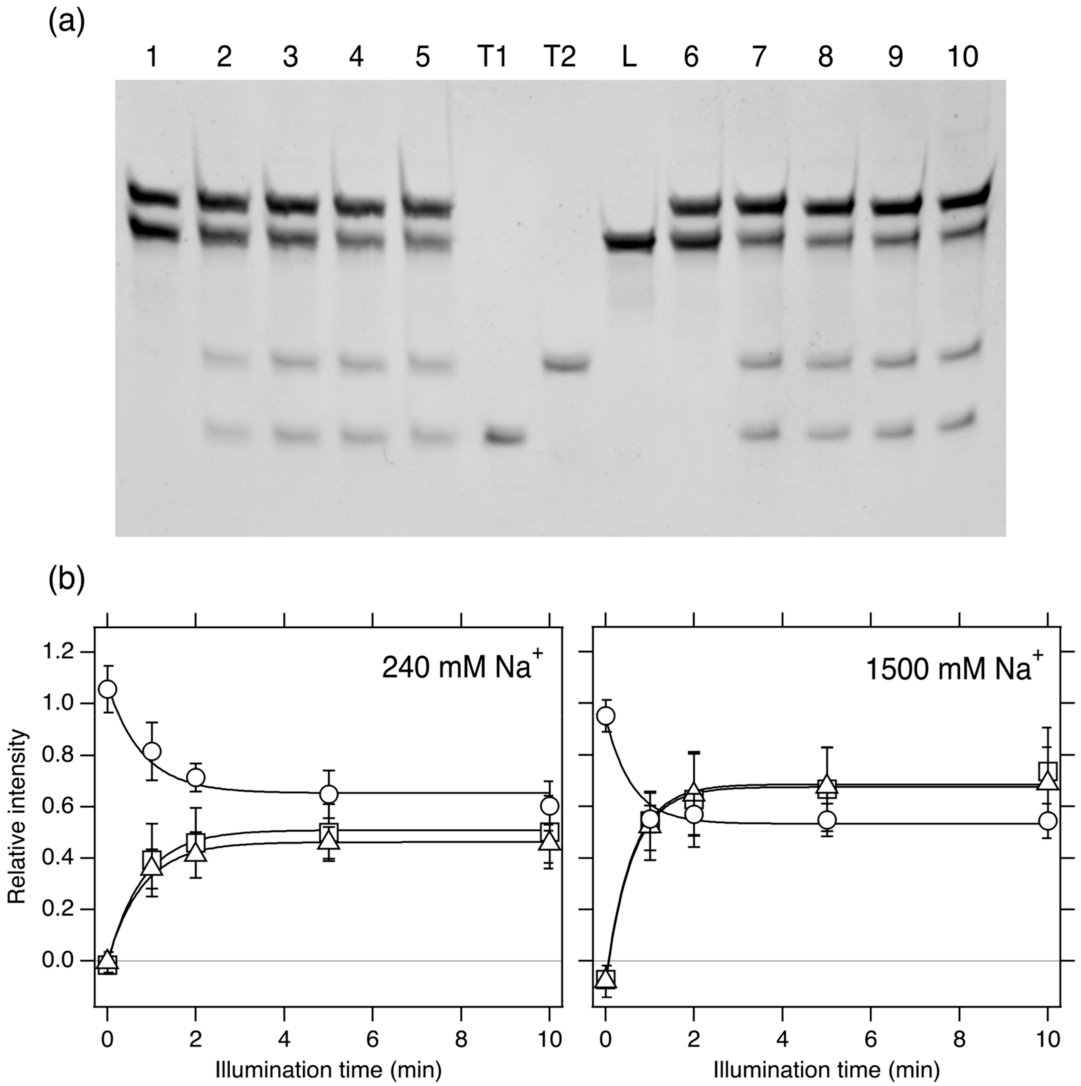

Figure 3. (a) Illumination time dependence of TDP repair by UV1C. UV1C and TDP in the presence of 240 mM (lanes 1−5) and 1500 mM (lanes 6−10) Na^+. Light illumination was carried out for 0 min (lanes 1 and 6), 1 min (lanes 2 and 7), 2 min (lanes 3 and 8), 5 min (lanes 4 and 9), and 10 min (lanes 5 and 10). Lane T1, 50 pmol of T1; T2, 50 pmol of T2; and L, 50 pmol of LMP. A typical electrophoresis picture is shown. (b) Band intensity was plotted as a function of the illumination time. The intensities of the formed T1, T2, and remaining TDP bands of lanes 4−8 in (a) were estimated by taking the intensity of the bands of 50 pmol of T1, T2, and LMP as 1, respectively. The relative intensities of T1, T2, and LMP are illustrated as squares, triangles, and circles, respectively. The data were fitted as a single exponential function.

to previous reports for the photolyase measurement with a slight modification.[17−19,23] FTIR spectra were measured before and after illumination with 295−370 nm light. Eight to ten spectra were averaged.

CPD photolyase from *Escherichia coli* was purified as previously reported.[17] TDP photorepair by CPD photolyase was measured by FTIR spectroscopy, as reported earlier but without CPD-containing DNA.[17−19,23]

3. RESULTS

3.1. Formation of TDP and Repair by UV1C. TDP formation and repair by UV1C were investigated in our system. Using DNA oligonucleotides and a xenon lamp, we attempted to synthesize TDP. There were many bands detected by ethidium bromide staining of urea polyacrylamide gel after UV irradiation (Figures S1 and S2). Of the DNA bands, 35-mer-long DNA was purified based on an assumption that the band was TDP (Figure S2). To investigate whether the purified DNA fragment was TDP, the DNA was subjected to repair by UV1C. The DNA was annealed with UV1C in the presence of 240 mM Na^+ and illuminated at 295−370 nm. The resultant sample contained 15-mer and 20-mer DNA bands (Figure S3). Based on their band intensity, it was estimated that approximately 50% was repaired after illumination for 10 min (Figure S4). Estimated from the band intensity of the remaining (uncut) TDP, TDP repair was about 30%. Although there was some discrepancy between the rates of formed T1 and T2 and remaining TDP, it was confirmed that the purified DNA contained TDP and that the UV1C repaired the TDP by illumination at 295−370 nm light (Figure S3).

Under our experimental conditions, a prolonged illumination time for more than 5 min did not affect the repair efficiency. The remaining DNA was either unrepaired TDP or contaminated DNA fragments. DNA was purified only by length; impurities were not checked. Although the purified DNA may have contained about half of the impurities, the DNA fragment was used as a TDP substrate in the following experiments.

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ACS Omega 2023, 8, 37274−37281

3.2. CD Spectroscopy of UV1C with the TDP Analogue. It has been shown that UV1C forms a parallel G-quadruplex from CD spectroscopy.[14] To determine the Na^+ concentration for subsequent FTIR spectroscopy, CD spectra were measured at various Na^+ concentrations. The measurements were initially conducted at Na^+ concentrations ranging from 0 to 500 mM. A spectral change was observed around 300 nm at 100−500 mM Na^+; therefore, measurements were extended to concentrations up to 1500 mM (Figure 1). In the absence of Na^+, the peaks appeared at 219 (+), 248 (−), and 278 (+) nm (Figure 1, black line). As the Na^+ concentration increased from 0 to 500 mM, a negative peak at 248 (−) nm slightly shifted to 246 (−) nm. Positive signals at 219 (+) and 278 (+) nm changed their intensity, with the former becoming smaller and the latter becoming larger. At 500−1500 mM Na^+ concentrations, a positive shoulder appeared at 298 nm. The peak at 278 (+) nm with a shorter wavelength shifted to 272 (+) nm. Difference spectra were calculated with respect to the CD spectrum at 0 mM Na^+ (Figure 1, inset). There were clearer peaks at 217 (−), 242 (−), 262 (+), and 298 (+) nm, with the peak at 298 (+) nm appearing in the presence of more than 500 mM Na^+.

The appearance of bands at 242 (−) and 262 (+) nm upon increasing Na^+ concentrations (Figure 1, inset) indicates the formation of parallel G-quadruplexes. On the other hand, the band at 298 (+) nm indicates the formation of antiparallel or hybrid G-quadruplexes.[22,24,25] This suggests that the complex of UV1C and LMP (TDP) forms G-quadruplexes with different structures depending on the Na^+ concentration. Assuming that Na^+-concentration-dependent CD spectral changes originated from two components, the components were separated (Figure S5a). The calculated difference CD spectra appearing at lower Na^+ concentrations peaked at 226 (+), 244 (−), and 268 (+) nm, while the signal appearing at higher Na^+ concentrations peaked at 248 (+), 278 (−), and 298 (+) nm. The relative ratios of the two signals at various Na^+ concentrations were estimated by taking the intensities of the two difference spectra at 1500 mM Na^+ as both 1 (Figure S5b).

From these difference CD spectra, "pure" CD spectra were estimated (Figure 2a). CD spectra were calculated by considering the reported absorbance coefficient of each G-quadruplex form.[22] Specifically, absorbance coefficients were selected, so that the sum of the ratios of formed G-quadruplexes would not exceed 1 (Figure 2b, broken line). Under this constraint, the absorption coefficients should exceed the values reported in the literature (Figure 2a). It may also be because the UV1C−LMP complex contains sequences that do not form the G-quadruplex.

The estimated CD spectrum at lower Na^+ concentrations peaked at 222 (+), 246 (−), and 272 (+) nm, suggesting the formation of parallel G-quadruplexes (Figure 2a, solid line). The CD spectrum at higher Na^+ concentrations peaked at 214 (+), 244 (−), 274 (+), and 297 (+) nm, which was a characteristic of hybrid G-quadruplexes. Using the CD spectra, the ratio of parallel and hybrid G-quadruplexes at each Na^+ concentration was estimated (Figure 2b).

The data were fitted to the theoretical formula, which indicated that 1.3 and 1.8 Na^+ were bound to form parallel and hybrid G-quadruplexes, respectively. The equilibrium constants K_1 and K_2 were calculated to be 1.0×10^{-3} $(mM)^{-1.3}$ and 3.2×10^{-5} $(mM)^{-1.8}$, respectively. Using formulae 3 and 4, the Na^+ concentrations at which half of the G-quadruplexes are formed were estimated to be 200 and 340 mM for parallel and hybrid G-quadruplexes, respectively. It seems reasonable that the CD spectra showed significant changes between 100 and 500 mM Na^+ (see Figure 1, green and yellow spectra).

Although there remain considerations on the quantity of molar coefficients of the CD spectra and the numbers of bound Na^+, the UV1C and LMP complex formed parallel G-quadruplexes at lower Na^+ concentrations and hybrid G-quadruplexes at higher concentrations.

3.3. Photorepair Activity of UV1C of Hybrid G-Quadruplexes. It was investigated whether hybrid G-quadruplexes have TDP repair activity. TDP repair by UV1C in the presence of 1500 mM Na^+ was compared with that in the presence of 240 mM Na^+ (Figure 3). The electrophoresis experiment showed that TDP repair reached saturation in about 5 min of illumination in the presence of 1500 mM Na^+ as well as the case for the 240 mM Na^+ condition (Figure 3b). The amount of formed T1 and T2 was about 70% of the standards. Estimated from remaining TDP, TDP cleavage occurred in about 50% of the standards. This suggests that TDP repair was more efficient for 1500 mM Na^+ samples, since the repair efficiency at 240 mM Na^+ was estimated to be 50% from formed T1 and T2 (Figures S4 and 3).

At 240 mM Na^+, the formed parallel and hybrid G-quadruplexes were estimated to be 45 and 20%, respectively, from a theoretical curve (Figure 2b). On the other hand, at 1500 mM Na^+ concentrations, the formed parallel and hybrid G-quadruplexes were estimated to be 47 and 49%, respectively (Figure 2b). UV1C and TDP were mixed at 1:1 stoichiometry in this experiment so that it is unlikely that one UV1C molecule would repair more than two TDP molecules. If only the UV1C of the parallel G-quadruplex structure was involved in the repair, the same amount of TDP would be repaired at 240 mM and 1500 mM Na^+ since the ratios of the formed parallel G-quadruplex are the same. However, this was not the case. Thus, it was concluded that both G-quadruplexes can repair TDP.

3.4. Light-Induced FTIR Difference Spectroscopy on the Photorepair of TDP by UV1C. Light-induced FTIR difference spectra of TDP repair by UV1C, whose Na^+ concentrations were 240 and 1500 mM, were measured (Figure 4a,b). The FTIR difference spectrum on TDP repair by CPD photolyase was also measured because it was reported that the TDP can be repaired by CPD photolyase (Figure 4c).[26] As shown in Figure 4d, there was a light-induced difference spectrum of CPD repair, including the phosphodiester bond between thymines, by *E. coli* CPD photolyase, which was reported previously.[19]

As shown in Figure 4c,d, three negative signals appeared at similar frequencies, at 1539, 1464 (1460), and 1398 (1396) cm^{-1}. These signals originated from CPD and were due to thymine dimer repair.[19] Therefore, the spectrum in Figure 4c was derived from the TDP cleavage by CPD photolyase. Spectral differences were observed in 1720−1600, ∼1300, and 1100−1000 cm^{-1} regions. The former originated from the amide-I vibration (C═O stretches from the peptide backbone), while the middle and latter regions are PO_2^- stretching vibrational regions. These results reflect conformational changes of both CPD photolyase and CPD (TDP) associated with binding and dissociation of the substrates.

The spectral shapes in Figure 4a,b were distinct from those in Figure 4c, for TDP repair by CPD photolyase, and they also differed from each other. Compared to the spectrum in Figure

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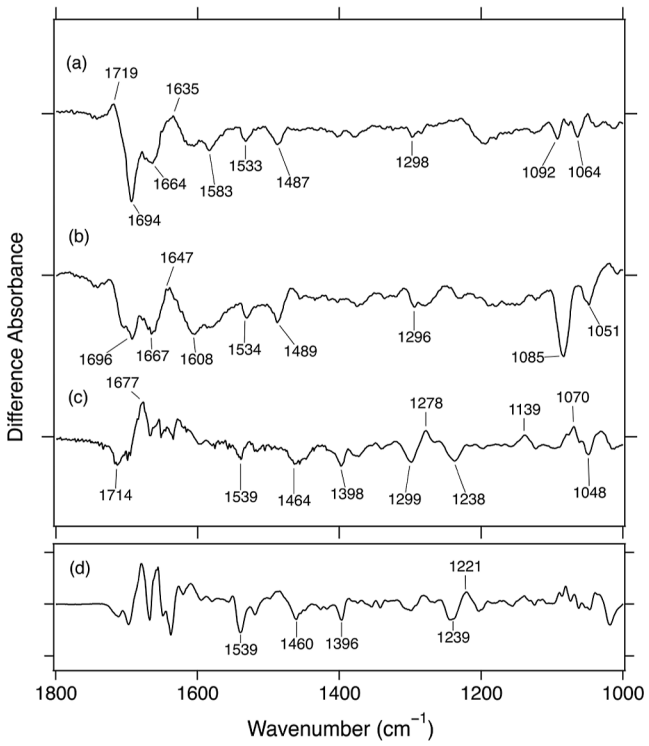

Figure 4. Light-induced FTIR difference spectra of the annealed sample of UV1C and TDP in the presence of 240 mM Na^+ (a) and 1500 mM Na^+ (b) and of the sample of *E. coli* CPD photolyase and TDP (c). (d) Light-induced FTIR difference spectrum of CPD repair (thymine dimers containing phosphodiester bonds in between) by *E. coli* CPD photolyase.[19] Reprinted in part with permission from *Biophysics* **2015, 11**, 39−45. Copyright 2015/The Biophysical Society of Japan.

4c, only one signal of a thymine dimer was observed at 1533 (1534) cm^{-1}. The band corresponding to 1464 cm^{-1} would be 1487 (1489) cm^{-1}, if they originate from the same vibrational signal, and the bands appeared at approximately 20 cm^{-1} higher-frequency region. There were no clear signals corresponding to 1398 cm^{-1}. Thus, these spectra may not reflect the photorepair of TDP by UV1C. However, the collected pre- and post-FTIR samples were electrophoresed, and truncated T1 and T2 were detected only in the post-FTIR samples (Figure S6), indicating that structural changes associated with TDP repair (dissociation) could occur after light illumination in the FTIR windows. Thus, these difference spectra originated from the photorepair of TDP by UV1C.

In the difference spectra of Figure 4a,b, differences were observed at 1725−1580 and 1100−1000 cm^{-1} regions. Differences in the Na^+ concentrations between the two sample conditions led to different ratios of the formed G-quadruplexes. This indicates that the difference spectra may include conformational changes in the UV1C enzymes and structural differences in TDP that binds to UV1C. The large signal at 1085 (−) cm^{-1} in the 1500 mM Na^+ sample (Figure 4b) was assignable to PO^- stretches. The phosphate backbone of UV1C forming hybrid G-quadruplexes may have shown large conformational changes associated with TDP cleavage and dissociation.

To summarize the results of light-induced FTIR difference spectra: (i) the structural changes of UV1C of parallel and hybrid G-quadruplexes are different and (ii) the difference spectra also included structural changes of the enzymes, CPD photolyase, and UV1C.

4. DISCUSSION

In this study, spectroscopic analyses of TDP repair by UV1C were performed. Prior to spectroscopic measurements, the results reported by Sen and Chinnapen[8] were reproduced in our experimental system. CD spectroscopy revealed Na^+-concentration-dependent spectral changes that reflect the G-quadruplex structures of the UV1C-TDP analogue (LMP) complexes, including parallel and hybrid G-quadruplexes. The light-induced FTIR difference spectra of UV1C- and TDP-annealed samples showed significant differences among UV1C with 240 and 1500 mM Na^+ and CPD photolyase, indicating that the conformational changes associated with TDP repair may differ among the enzymes.

4.1. Structures of UV1C Presumed by CD Spectra. Various sequences were reported that change the G-quadruplex structure formed depending on the cation concentration and type (reviewed in refs 27 and 28). It was found that UV1C with LMP also changed its structure in a Na^+-concentration-dependent manner. We fitted the Na^+-dependent conformational changes by assuming a two-state transition. Although we could not rule out the possibility that there are equilibrated multiple states at higher Na^+ concentrations, for example, antiparallel and hybrid G-quadruplexes. Here, we assumed a one-state hybrid G-quadruplex, at higher Na^+ concentrations. Conversely, this experiment did not provide relatively more accurate ratios of parallel and hybrid G-quadruplexes. Recently, it was reported that sorafenib binds to a groove of parallel G-quadruplex at the promoter region of the human c-myc gene.[29] If sorafenib only recognizes and binds to parallel G-quadruplexes and if such agents exist, we could more accurately estimate the ratios of parallel and hybrid G-quadruplexes.

It was suggested that both UV1C structures of parallel and hybrid G-quadruplexes have TDP repair activities. Parallel G-quadruplexes reportedly function as antennas, absorbing 310 nm light at UV1C. It is possible that a hybrid G-quadruplex may function as an antenna as well.

The structure of the hybrid form was estimated based on the parallel G-quadruplex structure that was previously reported.[12] Since both G-quadruplex structures have repair activity upon illumination with light >300 nm, it was considered that the relative positions of the thymine dimers and G-quadruplexes were similar; Sen and Chinnapen reported that TDP and UV1C interact through hydrogen bonds between bases that are not thymine dimers, and this interaction would also be conserved between them. It was hypothesized that some of the chains forming the G-quadruplexes are interchanged. A speculated schematic model is shown in Figure 5.

4.2. Environments of the CPD Moiety Bound to UV1C. In the FTIR difference spectra associated with repair (cleavage) of the CPD moiety in the TDP by UV1C, the infrared signals originating from the thymine dimer were not clearly observed (Figure 4). This suggests that the CPD moiety was exposed in solution, leading to the formation of various hydrogen bonds with water molecules, and consequently the signals did not appear at specific frequencies. This result supports the structure shown by Sen et al. (Figure 5a). This is in contrast with the way CPD is bound in the binding pocket of CPD photolyases.[30,31] It may not be essential for CPD repair to be in a fixed hydrogen bonding environment or a

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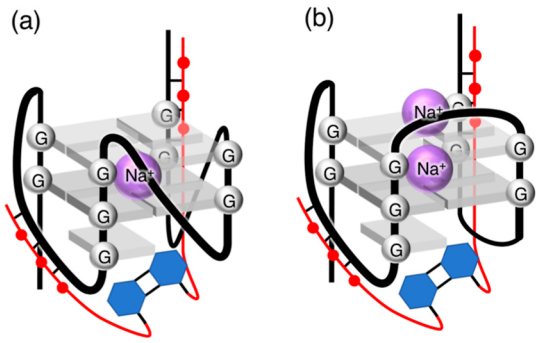

Figure 5. UV1C−TDP complex at lower Na^+ concentrations (a) and the speculated complex structure at higher Na^+ concentrations (b). The blue hexagons connected by two red lines represent the CPD in TDP.

hydrophobic environment. In fact, it has been reported that the efficiency of CPD repair in DNA strands by electron transfer from guanine is similar for single- and double-strand DNAs.[7] For CPD repair, the environment of the CPD itself might not be as important as the fixed positions between the CPD and the electron donor.

4.3. FTIR Difference Spectra Showing Photorepair of TDP by Different Enzymes. The FTIR difference spectra upon TDP photorepair showed different characteristics depending on the three enzymes used: UV1C with mainly parallel G-quadruplexes, UV1C with a higher ratio of hybrid G-quadruplexes, and CPD photolyase. Our previous photorepair experiments with 1:1 stoichiometry of photolyase and damaged DNA showed structural changes on the enzyme due to binding and dissociation with the substrate.[17,32] Therefore, the results indicate that the signals reflect conformational changes in the enzymes that reflect different conformations, even though the phenomena of TDP repair and dissociation of DNA strands are the same.

4.3.1. Binding and Repair of TDP by UV1C with Different G-Quadruplexes. As shown above, UV1C formed parallel and hybrid G-quadruplexes at lower and higher Na^+ concentrations, respectively. Reflecting their different ratios, the FTIR spectral shapes of the two structures were different, especially in the 1700−1500 and 1200−1100 cm^{-1} regions, the former in the C═O and C═N stretching and the latter in the phosphate PO^- stretching vibration regions, respectively. It was presumed that the structural changes in TDP (and repaired DNA) and UV1C were very different. The signals from PO^- stretches appeared more largely at the negative side for the UV1C and TDP with 1500 mM Na^+. Because no phosphate groups were lost during photorepair, it was thought that this reflects a change in the environments of the phosphate groups. Given that the substantial negative signal originates from the prerepair state, it is likely that either the phosphate backbone of TDP or UV1C experienced significant perturbation upon binding.

4.3.2. Comparison of Repair Spectra of TDP and CPD (Including Phosphodiester Bonds) by CPD Photolyase. The FTIR spectra upon TDP/CPD repair by CPD photolyase were similar in the 1500−1300 cm^{-1} region. The signals in the region were presumably due to the cleavage of thymine dimers. This indicates that the environment before and after the thymine dimer repair are similar, regardless of phosphodiester bonds between the thymine dimers. On the other hand, differences were observed in the 1750−1550 and 1300−1000 cm^{-1} regions. The former reflects the signal of the photolyase proteins, while the latter reflects DNA phosphate backbone changes. In other words, the conformations of proteins and substrate DNA associated with the binding and dissociation of damaged DNA are different between the two. The presence or absence of phosphodiester linkages between thymine dimers was thought to be responsible for these changes, although sequence differences and the presence or absence of phosphodiester linkages may influence the substrate DNA.

5. CONCLUSIONS

In this work, the Na^+-dependent G-quadruplexes of UV1C exhibit distinct conformations. FTIR results showed repair spectra reflecting the different conformations of the parallel and hybrid forms. In this experiment, Na^+ concentration dependence was examined, and effects of K^+ and pH of the solution also must be examined. As we have previously proposed a mechanism from detailed signal attribution by FTIR measurements of photolyases, UV1C will also provide structural information through the preparation of isotopically labeled samples. Additionally, this technique would be used for measuring the LDP repair by UV1C to separate substrate repair from dissociation. UV1C can be synthesized at arbitrary scales so that UV1C would serve as a model system for DNA photorepair.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c05083.

Urea-PAGE analyses to confirm UV irradiation conditions for TDP synthesis, to confirm if TDP have been purified, to confirm TDP repair by UV1C with and without light and Na^+, to confirm light conditions for TDP repair of UV1C; calculated difference CD spectra; and electrophoresis of samples before and after FTIR measurements (PDF)

Accession Codes

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Author Contributions

T.I. designed the research plan under the supervision of H.K. T.I., Y.K., and I M.M.W. conducted the experiments. T.I. wrote the manuscript with consensus from all authors. All authors have given approval to the final version of the manuscript.

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■ ABBREVIATIONS

DNAzyme, deoxyribozyme; UV, ultraviolet; CPD, cyclobutane pyrimidine dimer; G-quadruplex, guanine quadruplex; FTIR, Fourier transform infrared; (6-4)PP, pyrimidine-pyrimidone (6-4) photoproduct; TDP, thymine dimer-containing primer; FAD, flavin adenine dinucleotide; PAGE, polyacrylamide gel electrophoresis; CD, circular dichroism; LDP, ligated dimer primer; LMP, ligated monomeric primer

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