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Journal of Photochemistry and Photobiology A: Chemistry 197 (2008) 206–212
Journal of Photochemistry and Photobiology A:Chemistry
www.elsevier.com/locate/jphotochem

Intermolecular vs. intramolecular photoinduced electron transfer from nucleotides in DNA to acridinium ion derivatives in relation with DNA cleavage

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Received 8 November 2007; received in revised form 14 December 2007; accepted 26 December 2007
Available online 5 January 2008

Abstract

Photoirradiation of various 10-methylacridinium ions ($AcrR^+$, R = H, ^{i}Pr, and Ph) intercalated in DNA results in ultrafast intramolecular electron transfer, followed by rapid back electron transfer between $AcrR^+$ and nucleotides in DNA. The electron-transfer dynamics in DNA were monitored by femtosecond time-resolved transient absorption spectroscopy. Both acridinyl radical and nucleotide radical cations, formed in the photoinduced electron transfer in DNA, were successfully detected in an aqueous solution. These transient absorption spectra were assigned by the comparison with those of DNA nucleotide radical cations, which were obtained by the intermolecular electron-transfer oxidation of nucleotides with the electron-transfer state of 9-mesityl-10-methylacridinium ion ($Acr^{\bullet}$–$Mes^{\bullet+}$) produced upon photoexcitation of Acr^+–Mes. Photoinduced cleavage of DNA with various acridinium ions ($AcrR^+$, R = H, ^{i}Pr, Ph, and Mes) has also been examined by agarose gel electrophoresis, which indicates that the rapid intramolecular back electron transfer between acridinyl radical and nucleotide radical cation in DNA suppresses the DNA cleavage as compared with the intermolecular electron-transfer oxidation of nucleotides with $Acr^{\bullet}$–$Mes^{\bullet+}$.

Keywords: DNA; Electron transfer; Nucleotide; Radical; Laser flash photolysis

1. Introduction

The DNA double helix consists of a linear array of π-stacked, aromatic heterocyclic nucleobases within a polyanionic sugar-phosphates backbone, providing a unique medium for investigation of electron-transfer reactions [1–12]. Recent investigations of the dynamics of photoinduced electron transfer in DNA have employed probe chromophores that are π-stacked with an adjacent base pair [13–15]. For example, the dynamics of charge separation and charge recombination in DNA have been examined using a chromophore as a linker of two complementary oligonucleotides containing donor nucleotides [13]. Alternatively, noncovalently bound species (DNA intercalators) have also been used as chromophores in DNA to initiate photoinduced electron transfer between nucleotides in DNA [16,17]. Acridinium ions are also intercalated into the DNA double helix [18,19]. The occurrence of photoinduced electron transfer from nucleotides to singlet excited state of various acridinium ions has been confirmed by fluorescence quenching experiment and emission decay profiles [19,20]. Acridinium ions are known to have the small reorganization energy (λ) of electron transfer [21], which results in efficient electron transfer [22,23]. The observation of both radical anions of electron acceptors and radical cations of donor DNA bases provides a method for investigation of the dynamics of charge separation, charge recombination, and hole migration processes in DNA. However, there has so far been no report on direct observation of nucleotide radical cations in DNA by laser flash photolysis measurements because of the lack of reference for assignment and suitable chromophores for detection. On the other hand, the DNA cleavage results from the electron-transfer oxidation of DNA bases, followed by hole transfer to guanine part in vivo, which is known to be most readily oxidized among four DNA bases [24–31]. Although extensive efforts have so far been made to detect guanine radical cation [32,33], there has been no report on direct observation of radical cations

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1010-6030/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.jphotochem.2007.12.024

of the other DNA bases, i.e., adenine, cytosine, and thymine [34].

We report herein ultrafast photoinduced electron transfer and rapid back electron transfer between nucleotides and intercalated acridinium ions ($AcrR^+$, R = H, ^{i}Pr, and Ph) in DNA. Both acridinyl radical and nucleotide radical cation produced by the photoinduced electron-transfer reactions in DNA have been detected by femtosecond laser flash photolysis measurements. These transient absorption spectra of oxidized DNA by photoexcited acridinium ions in DNA were assigned by the comparison with those of DNA nucleotide radical cations, which were obtained independently by intermolecular electron-transfer oxidation of DNA bases with the electron-transfer state of a donor–acceptor dyad, 9-mesityl-10-methylacridinium ion ($Acr^{\bullet}$–$Mes^{\bullet+}$), produced upon photoexcitation of Acr^+–Mes. We also report the photoinduced DNA cleavage activity of $AcrR^+$.

2. Experimental

2.1. Materials

Calf-thymus deoxyribonucleic acid, sodium salt (CT DNA) was purchased from Sigma Chem. Co., USA. Stock solution of DNA (18 mg in 25 mL sol.) was prepared by dissolution overnight in 5 mmol dm^{-3} Tris–HCl buffer (pH 7.0) containing 5 mmol dm^{-3} sodium sulfate (Na_2SO_4). Supercoiled DNA, pBR322 was purchased from Wako Pure Chemical Industry, Japan. 9-Mesityl-10-methylacridinium (Acr^+–Mes), 10-methylacridinium ($AcrH^+$), and 9-phenyl-10-methylacridinium ($AcrPh^+$) perchlorate salts were purchased from Tokyo Kasei Organic Chemicals. 9-*iso*-Propyl-10-methylacridinium (Acr^iPr^+) perchlorate salt was prepared by the reported procedures [21,22b]. Purification of water (18.3 MΩ cm) was performed with a Milli-Q system (Millipore; Milli-RO 5 plus and Q plus).

2.2. Femtosecond laser flash photolysis

Ultrafast transient absorption spectroscopy experiments were conducted using an ultrafast source: Integra-C (Quantronix Corp.), an optical parametric amplifier: TOPAS (Light Conversion Ltd.) and a commercially available optical detection system: Helios provided by Ultrafast Systems LLC. The source for the pump and probe pulses were derived from the fundamental output of Integra-C (780 nm, 2 mJ/pulse and fwhm = 130 fs) at a repetition rate of 1 kHz. 75% of the fundamental output of the laser was introduced into TOPAS which has optical frequency mixers resulting in tunable range from 285 to 1660 nm, while the rest of the output was used for white light generation. Prior to generating the probe continuum, a variable neutral density filter was inserted in the path in order to generate stable continuum, then the laser pulse was fed to a delay line that provides an experimental time window of 3.2 ns with a maximum step resolution of 7 fs. In our experiments, a wavelength between 350 and 450 nm of TOPAS output, which is fourth harmonic of signal or idler pulses, was chosen as the pump beam. As this TOPAS output consists of not only desirable wavelength but also unnecessary wavelengths, the latter was deviated using a wedge prism with wedge angle of 18°. The desirable beam was irradiated at the sample cell with a spot size of 1-mm diameter where it was merged with the white probe pulse in a close angle (<10°). The probe beam after passing through the 2-mm sample cell was focused on a fiber optic cable that was connected to a CCD spectrograph for recording the time-resolved spectra (410–800 nm). Typically, 2500 excitation pulses were averaged for 5 s to obtain the transient spectrum at a set delay time. Kinetic traces at appropriate wavelengths were assembled from the time-resolved spectral data. All measurements were conducted at room temperature, 295 K.

2.3. Nanosecond laser flash photolysis

For the nanosecond laser flash photolysis experiments, degassed aqueous buffer solutions were excited by Nd:YAG laser (Continuum, SLII-10, 4–6 ns fwhm, 30 mJ/pulse) at 355 nm. Time courses of the transient absorption spectra were measured by using a continuous Xe-lamp (150 W) and an In GaAs-PIN photodiode (Hamamatsu 2949) as a probe light and a detector, respectively. The output from the photodiodes and a photomultiplier tube was recorded with a digitizing oscilloscope (Tektronix, TDS3032, 300 MHz). The transient spectra were recorded using fresh solutions in each laser excitation. All experiments were performed at 298 K.

2.4. Fluorescence lifetime measurements

Time-resolved fluorescence spectra were measured by a Photon Technology International GL-3300 with a Photon Technology International GL-302, nitrogen laser/pumped dye laser system, equipped with a four channel digital delay/pulse generator (Stanford Research System Inc. DG535) and a motor driver (Photon Technology International MD-5020). The excitation wavelength was 420 nm using POPOP (Wako Pure Chemical Ind. Ltd., Japan) as a laser dye.

2.5. DNA cleavage

3 μL of aqueous solutions of DNA pBR322 (0.51 μg μL^{-1}) was dried in vacuo. Typically, 30 μL of deaerated aqueous buffer solutions (10 mmol dm^{-3} CH_3COOH/KOH (pH 5.0) or 10 mmol dm^{-3} KH_2PO_4/NaOH (pH 7.0)) of $AcrR^+$ (1.0×10^{-4} mol dm^{-3}) and dried DNA was mixed in microtest tubes under N_2. Deaerated aqueous buffer solutions were prepared after three freeze-pump-thaw cycles. Samples were incubated under irradiation with a monochromatized light (λ = 360 nm) from a Shimadzu RF-5300PC spectrophotometer at 298 K. The 3 μL of aqueous solutions of DNA pBR322 (0.051 μg μL^{-1}) were diluted by adding 27 μL of water, then mixed with 3 μL of loading buffer (0.1% bromophenol blue and 3.75% ficol in TAE buffer) and loaded onto 1.4% agarose gel. The gel was run at a constant voltage of 130 V for 50 min in TAE buffer using a Nihon Eido electrophoresis kit, then washed with distilled water, soaked into 0.1% ethidium bro-

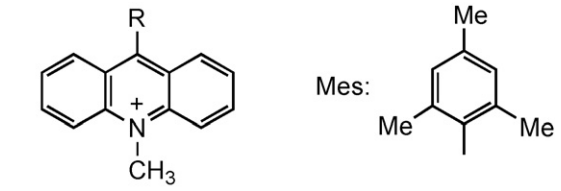


Fig. 1. Structure of 10-methylacridinium ions used in this study.

mide aqueous solution, visualized under a UV transilluminator, and photographed using a digital camera.

3. Results and discussion

3.1. Photoinduced electron transfer and back electron transfer between DNA and 10-methylacridinium ions in DNA

10-Methylacridinium ions, $AcrH^+$, Acr^iPr^+, and $AcrPh^+$ (shown in Fig. 1) are known to be intercalated into the DNA double helix [18,19]. The singlet excited state of $AcrR^+$ can act as a strong oxidant (E_{red} = 2.47 V ($^1AcrH^{+*}$), 2.02 V ($^1Acr^iPr^{+*}$), and 1.93 V ($^1AcrPh^{+*}$) vs. SCE) in an aqueous solution [35,36]. When $AcrR^+$ is intercalated into DNA, the one-electron reduction of $AcrR^+$ becomes easier as compared to that in an aqueous solution [18]. As a result, the reduction potentials of the singlet excited states of $AcrR^+$ in DNA are shifted to positive direction: 2.66 V (vs. SCE, $^1AcrH^{+*}$), 2.16 V (vs. SCE, $^1Acr^iPr^{+*}$), and 2.10 V (vs. SCE, $^1AcrPh^{+*}$), respectively [36]. Since the one-electron reduction potential of $^1AcrR^{+*}$ is more positive than the one-electron oxidation potential of all nucleotides, electron transfer from nucleotides to the singlet excited state of $AcrR^+$ is energetically feasible [20]. Thus, the fluorescence of $^1AcrR^{+*}$ are quenched via electron transfer by addition of DNA nucleotides [19,20]. However, no transient absorption spectrum was observed upon nanosecond laser excitation of a deaerated deionized aqueous solution of $AcrR^+$ in the presence of DNA. This indicates that rapid back electron transfer occurs following photoinduced electron transfer from DNA to $^1AcrR^{+*}$ at the time scale of femtosecond. Thus, the photoinduced electron transfer between $AcrR^+$ and DNA bases in DNA was studied by femtosecond laser flash photolysis (vide infra).

Femtosecond laser excitation at 380 nm of a deaerated aqueous solution containing CT DNA (6.6×10^{-3} mol dm^{-3}) [37] and $AcrH^+$ (6.1×10^{-4} mol dm^{-3}) results in appearance of the transient absorption spectra as shown in Fig. 2a. The transient absorption bands may be assigned to $AcrH^{\bullet}$ and DNA radical cation, both of which are produced by photoinduced electron transfer from nucleotides in DNA to the singlet excited state of $AcrH^+$. The absorption band due to $AcrH^{\bullet}$ is observed at 520 nm [21,38,39]. Free $AcrH^+$, which is not inserted in DNA, also exists in an aqueous buffer solution under these experimental conditions. The transient absorption around 680 nm, which remains at 110 ps after laser excitation, is assigned to the singlet–singlet absorption of $AcrH^+$. Similar transient absorption bands are observed for the case of Acr^iPr^+ (Fig. 2b). In the case of $AcrPh^+$ (Fig. 2c), the absorption band assigned to singlet–singlet absorption of $AcrPh^+$ [40] was not seen, because the absorption band due to singlet–singlet absorption of $AcrPh^+$

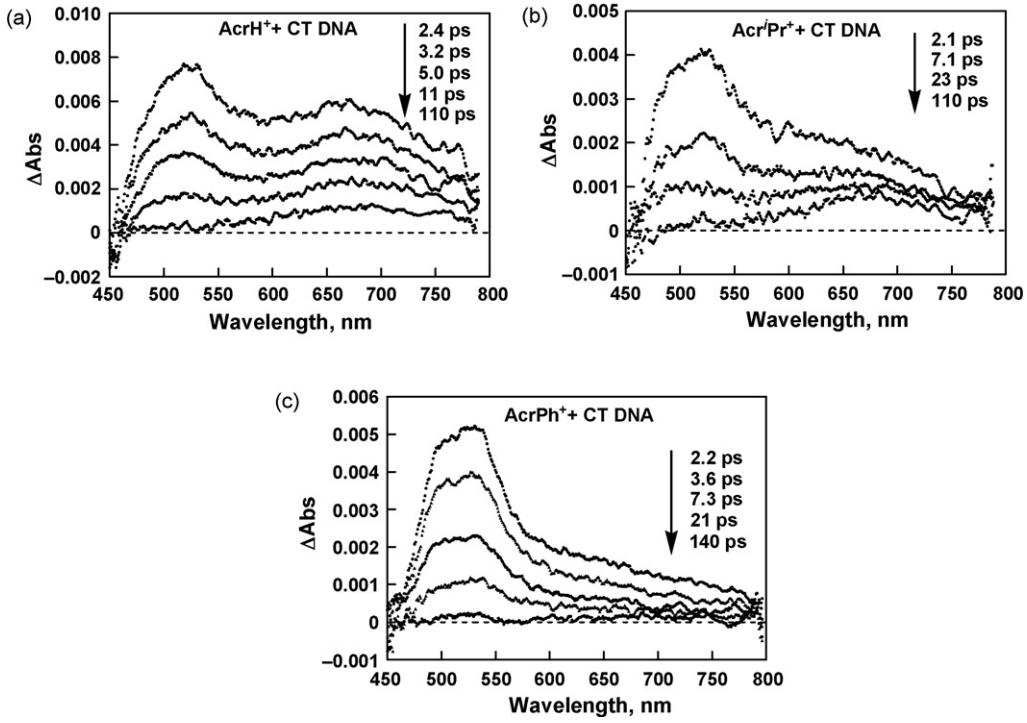


Fig. 2. Transient absorption spectra observed by the femtosecond laser excitation (λ = 380 nm) of deaerated aqueous solutions containing CT DNA (6.6×10^{-3} mol dm^{-3}) and $AcrR^+$: (a) $AcrH^+$ (6.1×10^{-4} mol dm^{-3}), (b) Acr^iPr^+ (1.8×10^{-4} mol dm^{-3}), and (c) $AcrPh^+$ (4.5×10^{-4} mol dm^{-3}) at 295 K.

is less intense and the band shape of the singlet–singlet absorption is different as compared to that of $AcrH^+$. The intensities of these transient absorptions due to $^1AcrR^{+}*$ are scarcely changed in this time scale because the lifetimes of these singlet excited states are longer than the time scale in Fig. 2. The fluorescence lifetimes of $AcrH^+$, Acr^iPr^+, and $AcrPh^+$ are determined as 31, 26, and 1.3 ns in deaerated buffer solutions, respectively (data not shown).

The transient absorption spectra of radical cations of DNA bases were independently observed by intermolecular electron-transfer oxidation of DNA bases with the electron-transfer state of a donor–acceptor dyad, Acr^+–Mes (the structure is shown in Fig. 1) [22,41–43]. No UV–vis absorption spectral change of Acr^+–Mes in buffer solution was observed upon addition of more than 30 equiv. of CT DNA [44]. Thus, there is no intercalation between a bulky Acr^+–Mes and double-stranded DNA because the dihedral angle between the Acr^+ and the Mes moieties of Acr^+–Mes is perpendicular [22].

Nanosecond laser excitation at 355 nm of a deaerated buffer solution of Acr^+–Mes results in formation of the long-lived electron-transfer state ($Acr^{\bullet}$–$Mes^{\bullet+}$) via photoinduced electron transfer from the Mes moiety to the singlet excited state of the Acr^+ moiety [22]. Since the one-electron reduction potential of $Acr^{\bullet}$–$Mes^{\bullet+}$ ($E_{red} = 1.88$ V vs. SCE), is more positive than the one-electron oxidation potential of all nucleotides, $E_{ox} = 1.07$ V (GMP: guanosine-5′-monophosphate), 1.18 V (AMP: adenosine-5′-monophosphate), 1.21 V (TMP: thymidine-5′-monophosphate), and 1.26 V (CMP: cytidine-5′-monophosphate), versus SCE, respectively [20], electron transfer from DNA nucleotides to the $Mes^{\bullet+}$ moiety in $Acr^{\bullet}$–$Mes^{\bullet+}$ is energetically feasible. The addition of GMP to a buffer solution of Acr^+–Mes at pH 2.0 and the laser photoirradiation result in formation of GMP radical cation ($GMP^{\bullet+}$: $\lambda_{max} = 510$ nm) [32,33,45]. The difference spectra obtained by subtracting the spectrum in the absence of GMP from those in the presence of GMP at pH 2.0 is shown in Fig. 3 where the absorption spectra of $GMP^{\bullet+}$ agree with those reported in literature [32,33].

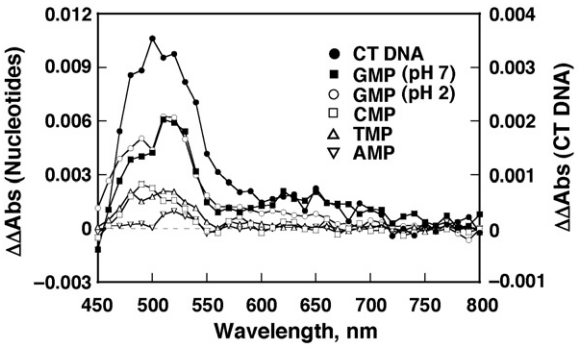


Fig. 3. Different transient absorption spectra of $(GMP\text{-}H)^{\bullet}$ (■, pH 7.0), $GMP^{\bullet+}$ (◯, pH 2.0), $CMP^{\bullet+}$ (□, pH 7.0), $TMP^{\bullet+}$ (△, pH 7.0), $AMP^{\bullet+}$ (▽, pH 7.0) and oxidized form of CT DNA (●, pH 7.0), obtained by subtracting the transient absorption spectra of Acr^+–Mes (6.0×10^{-5} mol dm^{-3}) in the absence of GMP from those in the presence of GMP (1.0×10^{-2} mol dm^{-3}), CMP (7.0×10^{-2} mol dm^{-3}), TMP (1.0×10^{-1} mol dm^{-3}), AMP (1.0×10^{-1} mol dm^{-3}), and CT DNA (1.0×10^{-3} mol dm^{-3}), respectively. All transient absorption spectra were measured at 250 μs after laser excitation at λ = 355 nm at 298 K.

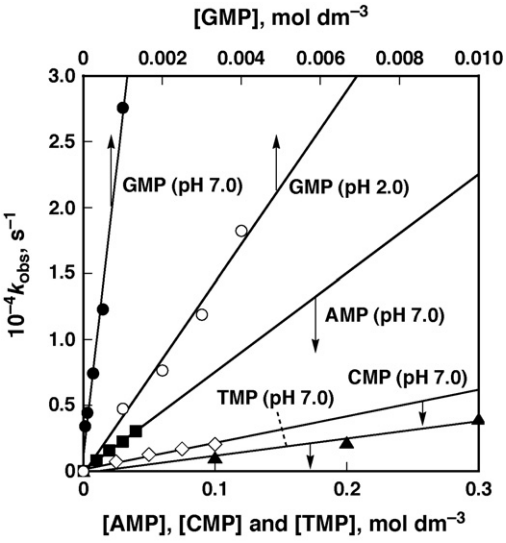
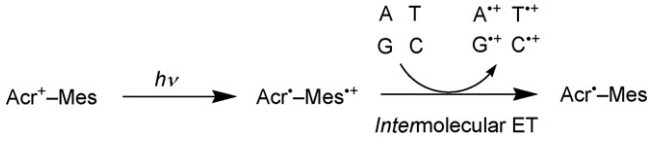


Fig. 4. Plots of the pseudo-first-order rate constants (k_{obs}) vs. [GMP] (◯, pH 2.0; ●, pH 7.0), [AMP] (■), [CMP] (◊) and [TMP] (▲) for the formation of nucleotides' radical cation in the photoreaction of Acr^+–Mes with nucleotides in 5 mmol dm^{-3} KCl/HCl (pH 2.0) or Tris–HCl (pH 7.0) buffer at 298 K.

The formation rate of $GMP^{\bullet+}$ obeyed pseudo-first-order kinetics and the pseudo-first-order rate constant (k_{obs}) increases linearly with increasing concentration of GMP at pH 2.0 as shown in Fig. 4. The second-order rate constant (k_{et}) of electron transfer from GMP to the $Mes^{\bullet+}$ moiety of $Acr^{\bullet}$–$Mes^{\bullet+}$ is determined as 4.3×10^7 dm^3 mol^{-1} s^{-1} in the buffer solution at 298 K. At pH 7.0, a transient absorption at long wavelength region ca. at 650 nm appears because of the deprotonation of $GMP^{\bullet+}$ $(GMP\text{-}H)^{\bullet}$ [33] as also shown in Fig. 3.

Transient absorption spectra of radical cations of CMP, TMP and AMP are also detected in the photoirradiation of Acr^+–Mes in the presence of CMP, TMP and AMP (Fig. 3 and Scheme 1). The absorption maxima of the radical cations of CMP, TMP and AMP in the range from 450 to 550 nm are similar to that of $GMP^{\bullet+}$, although the absorption intensity varies depending on DNA nucleotides. The k_{et} values of electron transfer from GMP (pH 7.0 and 2.0), CMP (pH 7.0), TMP (pH 7.0) and AMP (pH 7.0) to the $Mes^{\bullet+}$ moiety of $Acr^{\bullet}$–$Mes^{\bullet+}$ are determined as listed in Table 1 [34]. The rate of electron-transfer oxidation of GMP is two to three orders of magnitude faster than those of other nucleotides at pH 7.0. To clarify the site of electron-transfer oxidation of stacked double helix DNA, transient absorption measurements were also carried out using CT DNA with Acr^+–Mes. CT DNA was also efficiently oxidized by the electron-transfer state of Acr^+–Mes. The transient



Scheme 1.

Table 1
One-electron oxidation potentials (E_{ox}) of nucleotides and rate constants of electron-transfer reactions (k_{et}) at pH 7.0

DNA base	E_{ox} (V vs. SCE)	k_{et} ($dm^3\ mol^{-1}\ s^{-1}$)
GMP	1.07	2.7×10^8
AMP	1.18	7.6×10^5
TMP	1.21	1.2×10^5
CMP	1.26	2.2×10^5
CT DNA		4.8×10^7

Table 2
Rate constants of photoinduced electron transfer (PET) and back electron transfer (BET) and ratios of decay components

Intercalator	k_{PET} (s^{-1})	k_{BET} (s^{-1})
$AcrH^+$	$>3.0 \times 10^{12}$	5.8×10^{11} (76%)
Acr^iPr^+	$>3.0 \times 10^{12}$	4.5×10^{11} (70%)
$AcrPh^+$	$>3.0 \times 10^{12}$	5.0×10^{11} (70%)

absorption spectrum of oxidized CT DNA measured at pH 7.0 is similar to that of $(GMP\text{-}H)^\bullet$ (Fig. 3). The k_{et} value of the electron-transfer oxidation of CT DNA was determined as $4.8 \times 10^7\ dm^3\ mol^{-1}\ s^{-1}$. The electron-transfer oxidation of CT DNA may occur mainly at the guanine unit, because the k_{et} value of GMP is much larger than those of other DNA bases (Table 1). This may be the reason why the transient absorption spectrum of oxidized CT DNA at pH 7.0 is similar to that of $(GMP\text{-}H)^\bullet$.

The transient absorption spectrum in Fig. 2c is similar to those of DNA base radical cations in Fig. 3. The difference in the absorption maximum in the range from 450 to 550 nm in Fig. 2c from those of DNA base radical cations in Fig. 3 results from the overlap of the absorption band due to $AcrPh^\bullet$, which has the absorption maximum at 520 nm [46]. The absorption band of $AcrR^\bullet$ (R = H, ^{i}Pr) is also observed at 520 nm [21,38,39,47]. Thus, the transient absorption spectra in Fig. 2 are the superposition of those of DNA radical cations ($DNA^{\bullet+}$) and $AcrR^\bullet$ [48]. At the time scale in Fig. 2 (0–140 ps), no deprotonation of guanine radical cation occurs, because the deprotonation rate constant was reported to be $1.8 \times 10^7\ s^{-1}$ (the corresponding lifetime of the radical cation is 56 ns) at pH 7.0 [32].

The rate constant (k_{PET}) of photoinduced electron transfer from DNA bases to various $^1AcrR^+$* in CT DNA is estimated as faster than $3.0 \times 10^{12}\ s^{-1}$, which is the limitation of our femtosecond laser flash photolysis measurements. On the other hand, the decay process consists of at least two exponential components. The rate constants of the faster decay component (k_{BET1}) for $AcrH^+$, Acr^iPr^+, and $AcrPh^+$ are determined as $5.8 \times 10^{11}\ s^{-1}$ (76%), $4.5 \times 10^{11}\ s^{-1}$ (70%), and $5.0 \times 10^{11}\ s^{-1}$ (70%), respectively. These results are summarized in Table 2.

Acridinium ions are known to be intercalated between GC base pairs more easily than AT base pairs [19]. Thus, the main decay component with the faster rate may correspond to the back electron transfer from $AcrR^\bullet$ to the radical cation of GC base pair to which $AcrR^+$ is inserted, whereas the minor decay component with the slower rate may result from the back electron transfer from $AcrR^\bullet$ to the radical cation of an AT base pair. The slower back electron transfer from $AcrR^\bullet$ to the radical cation of an AT base pair may result from the weaker interaction of $AcrR^\bullet$ with the radical cation of an AT base pair as compared with that of a GC base pair.

Judging from the fast electron-transfer rate ($k_{PET} > 3 \times 10^{12}\ s^{-1}$) with a large driving force of photoinduced electron transfer from DNA bases to $^1AcrH^+$* [1.59 eV for photoinduced electron transfer from GMP (E_{ox} = 1.07 V vs. SCE) [20] to $^1AcrH^+$* (E_{red} = 2.66 V vs. SCE)], the λ value of PET in DNA may be estimated as ca. 1.6 eV. In such a case, the back electron transfer from $AcrH^\bullet$ to the radical cations of DNA bases is in the Marcus normal region, because the driving force of back electron transfer (1.18 eV) is smaller than the reorganization of electron transfer (1.6 eV), when the rate of back electron transfer is slower than the PET rate. The k_{BET1} value of $AcrH^+$ ($5.8 \times 10^{11}\ s^{-1}$) is the largest, resulting from the strongest interaction with DNA bases because of the least steric effect on the intercalation into DNA as compared with other $AcrR^+$ compounds with large substituents [19].

3.2. DNA cleavage induced by inter- vs. intramolecular photoinduced electron transfer to 10-methylacridinium ion

We also examined the DNA-photocleavage activity of $AcrH^+$, Acr^iPr^+, and $AcrPh^+$ as compared with Acr^+–Mes. The agarose gel electrophoresis was performed after 5 min photoirradiation of pBR322 with the monochromatized light (λ = 360 nm) in the presence of $AcrR^+$ as shown in Fig. 5. Although all DNA nucleotides can be oxidized by the $Mes^{\bullet+}$ moiety of $Acr^\bullet$–$Mes^{\bullet+}$ (Fig. 3), the largest k_{et} value of the electron-transfer oxidation of GMP together with the lowest oxidation potential of GMP

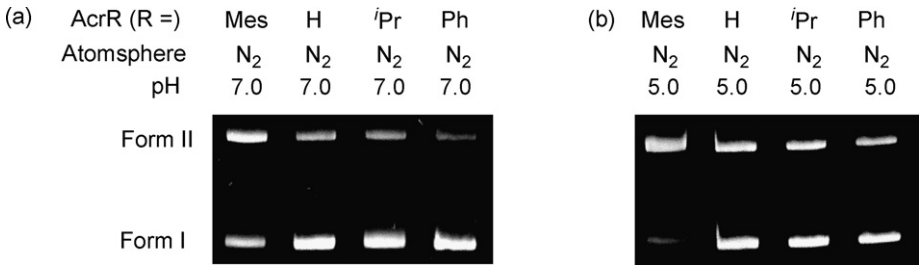


Fig. 5. Agarose gel electrophoresis of photoinduced cleavage of supercoiled pBR322 DNA (7.8×10^{-5} mol dm^{-3}) with 9-substituted acridinium ions ($AcrR^+$: 1.0×10^{-4} mol dm^{-3}) in (a) a nitrogen-saturated 10 mmol dm^{-3} KH_2PO_4/NaOH buffer (pH 7.0) and (b) a nitrogen-saturated 10 mmol dm^{-3} CH_3COOH/KOH buffer (pH 5.0) at 298 K after 5 min photoirradiation of monochromatized light (λ = 360 nm).

among nucleotides (vide supra) indicate that guanine is eventually oxidized in electron transfer from DNA to the $Mes^{\bullet+}$ moiety of $Acr^{\bullet}$–$Mes^{\bullet+}$, leading to the efficient DNA cleavage. The higher DNA cleavage activity at pH 5.0 as compared with that at pH 7.0 suggests that guanine radical cation has a higher reactivity for the DNA cleavage than the deprotonated radical judging from the pK_a value of guanine radical cation ($pK_a = 3.9$) [33].

The reactivity of DNA cleavage increases in the following order: $AcrPh^+ < AcrH^+$, $Acr^iPr^+ \ll Acr^+$–Mes. The low reactivity of $AcrH^+$, Acr^iPr^+, and $AcrPh^+$ for DNA cleavage in comparison with Acr^+–Mes results from the rapid back electron transfer from acridinyl radicals to nucleotides radical cations in DNA (vide supra) [49].

4. Conclusions

The transient absorption spectra of DNA nucleotides radical cations in DNA have been successfully detected in the electron-transfer oxidation of DNA with the singlet excited state of $AcrR^+$ ($AcrH^+$, Acr^iPr^+, and $AcrPh^+$) by femtosecond laser flash photolysis measurements. The results of agarose gel electrophoresis of photoinduced cleavage of DNA with various acridinium indicate that the rapid back electron transfer from $AcrR^{\bullet}$ to nucleotides radical cations in DNA suppresses the DNA cleavage.

Acknowledgments

This work was partially supported by a Grant in Aid (Nos. 19205019 and 19750034) from the Ministry of Education, Culture, Sports, Science and Technology, Japan (to S.F. and K.O.), and JSPS Fellowships (to M.T.).

References

[1] (a) R.E. Holmlin, P.J. Dandliker, J.K. Barton, Angew. Chem. Int. Ed. Engl. 36 (1997) 2714–2730;
(b) T.L. Netzel, in: V. Ramamurthy, K.S. Schanze (Eds.), Organic and Inorganic Photochemistry, vol.2, Marcel Dekker, New York, 1998, pp. 1–54.
[2] (a) J.K. Barton, C.V. Kumar, N.J. Turro, J. Am. Chem. Soc. 108 (1986) 6391–6393;
(b) M.D. Purugganan, C.V. Kumar, N.J. Turro, J.K. Barton, Science 241 (1988) 1645–1649;
(c) E.D.A. Stemp, J.K. Barton, Met. Ions Biol. Syst. 33 (1996) 325–365;
(d) D.B. Hall, R.E. Holmlin, J.K. Barton, Nature 382 (1996) 731–735;
(e) K.E. Erkkila, D.T. Odom, J.K. Barton, Chem. Rev. 99 (1999) 2777–2796.
[3] T.G. Drummond, M.G. Hill, J.K. Barton, J. Am. Chem. Soc. 126 (2004) 15010–15011.
[4] (a) C.J. Murphy, M.R. Arkin, N.D. Ghatlia, S. Bossmann, N.J. Turro, J.K. Barton, Proc. Natl. Acad. Sci. U.S.A. 91 (1994) 5315–5319;
(b) P.J. Dandliker, R.E. Holmlin, J.K. Barton, Science 275 (1997) 1465–1468.
[5] C.J. Burrows, J.G. Muller, Chem. Rev. 98 (1998) 1109–1152.
[6] B. Armitage, Chem. Rev. 98 (1998) 1171–1200.
[7] F.D. Lewis, in: V. Balzani (Ed.), Electron Transfer in Chemistry, vol. 3, Wiley-VCH, Weinheim, 2001, pp. 105–175.
[8] (a) B. Giese, Acc. Chem. Res. 33 (2000) 631–636;
(b) B. Giese, Annu. Rev. Biochem. 71 (2002) 51–70;
(c) B. Giese, J. Amaudrut, A.K. Kohler, M. Spormann, S. Wessley, Nature 412 (2001) 318–320;
(d) E. Meggers, A. Dussy, T. Schäfer, B. Giese, Chem. Eur. J. 6 (2000) 485–492.
[9] G.B. Schuster, Acc. Chem. Res. 33 (2000) 253–260.
[10] (a) K. Kawai, T. Takada, T. Nagai, X. Cai, A. Sugimoto, M. Fujitsuka, T. Majima, J. Am. Chem. Soc. 125 (2003) 16198–16199;
(b) T. Takada, K. Kawai, X. Cai, A. Sugimoto, M. Fujitsuka, T. Majima, J. Am. Chem. Soc. 126 (2004) 1125–1129;
(c) T. Takada, K. Kawai, M. Fujitsuka, T. Majima, J. Am. Chem. Soc. 128 (2006) 11012–11013.
[11] (a) S. Breeger, U. Hennecke, T. Carell, J. Am. Chem. Soc. 126 (2004) 1302–1303;
(b) C. Haas, K. Kraeling, M. Cichon, N. Rahe, T. Carell, Angew. Chem. Int. Ed. 43 (2004) 1842–1844.
[12] (a) T. Ito, S.E. Rokita, J. Am. Chem. Soc. 125 (2003) 11480–11481;
(b) T. Ito, S.E. Rokita, Angew. Chem. Int. Ed. 43 (2004) 1839–1842;
(c) T. Ito, A. Kondo, S. Terada, S. Nishimoto, J. Am. Chem. Soc. 128 (2006) 10934–10942.
[13] (a) F.D. Lewis, R.S. Kalgutkar, Y. Wu, X. Liu, J. Liu, R.T. Hayes, S.E. Miller, M.R. Wasielewski, J. Am. Chem. Soc. 122 (2000) 12346–12351;
(b) F.D. Lewis, R.L. Letsinger, M.R. Wasielewski, Acc. Chem. Res. 34 (2001) 159–170;
(c) F.D. Lewis, X. Liu, S.E. Miller, R.T. Hayes, M.R. Wasielewski, J. Am. Chem. Soc. 124 (2002) 14020–14026;
(d) F.D. Lewis, J. Liu, X. Zuo, R.T. Hayes, M.R. Wasielewski, J. Am. Chem. Soc. 125 (2003) 4850–4861.
[14] A. Harriman, Angew. Chem. Int. Ed. 38 (1999) 945–949.
[15] (a) C. Wan, T. Fiebig, S.O. Kelley, C.R. Treadway, J.K. Barton, A.H. Zewail, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 6014–6019;
(b) S.O. Kelley, J.K. Barton, Science 283 (1999) 375.
[16] G.D. Reid, D.J. Whittaker, M.A. Day, D.A. Turton, V. Kayser, J.M. Kelly, G.S. Baddard, J. Am. Chem. Soc. 124 (2002) 5518–5527.
[17] C. Dohno, E.D.A. Stemp, J.K. Barton, J. Am. Chem. Soc. 125 (2003) 9586–9587.
[18] S. Fukuzumi, M. Nishimine, K. Ohkubo, N.V. Tkachenko, H. Lemmetyinen, J. Phys. Chem. B 107 (2003) 12511–12518.
[19] J. Joseph, E. Kuruvilla, A.T. Achuthan, D. Ramaiah, G.B. Schuster, Bioconjugate Chem. 15 (2004) 1230–1235.
[20] S. Fukuzumi, H. Miyao, K. Ohkubo, T. Suenobu, J. Phys. Chem. A 109 (2005) 3285–3294.
[21] S. Fukuzumi, K. Ohkubo, T. Suenobu, K. Kato, M. Fujitsuka, O. Ito, J. Am. Chem. Soc. 123 (2001) 8459–8467.
[22] (a) S. Fukuzumi, H. Kotani, K. Ohkubo, S. Ogo, N.V. Tkachenko, H. Lemmetyinen, J. Am. Chem. Soc. 126 (2004) 1600–1601;
(b) H. Kotani, K. Ohkubo, S. Fukuzumi, J. Am. Chem. Soc. 126 (2004) 15999–16006;
(c) K. Ohkubo, T. Nanjo, S. Fukuzumi, Catal. Today 117 (2006) 356–361;
(d) K. Ohkubo, T. Nanjo, S. Fukuzumi, Bull. Chem. Soc. Jpn. 79 (2006) 1489–1500.
[23] M. Tanaka, K. Ohkubo, C.P. Gros, R. Guilard, S. Fukuzumi, J. Am. Chem. Soc. 128 (2006) 14625–14633.
[24] (a) Y. Yoshioka, Y. Kitagawa, Y. Takano, K. Yamaguchi, T. Nakamura, I. Saito, J. Am. Chem. Soc. 121 (1999) 8712–8719;
(b) K. Kino, I. Saito, H. Sugiyama, J. Am. Chem. Soc. 120 (1998) 7373–7374;
(c) I. Saito, M. Takayama, H. Sugiyama, K. Nakatani, J. Am. Chem. Soc. 117 (1995) 6406–6407;
(d) S. Matsugo, S. Kawanishi, K. Yamamoto, H. Sugiyama, I. Matsuura, I. Saito, Angew. Chem. Int. Ed. Engl. 30 (1991) 1351–1353;
(e) K. Ito, S. Inoue, K. Yamamoto, S. Kawanishi, J. Biol. Chem. 268 (1993) 13221–13227.
[25] R.A. Floyd, M.S. West, K.L. Eneff, J.E. Schneider, Arch. Biochem. Biophys. 273 (1989) 106–111.
[26] T.P.A. Devasagayam, S. Steenken, M.S.W. Obendorf, W.A. Schulz, H. Sies, Biochemistry 30 (1991) 6283–6289.
[27] P.M. Cullis, M.E. Malone, L.A. Merson-Davies, J. Am. Chem. Soc. 118 (1996) 2775–2781.
[28] M. Núñez, D.B. Hall, J.K. Barton, Chem. Biol. 6 (1999) 85–97.

[29] S. Boutorine, H. Tokuyama, M. Takasugi, H. Isobe, E. Nakamura, C. Helene, Angew. Chem. Int. Ed. Engl. 33 (1994) 2462–2465.
[30] Y.-Z. An, C.-H.B. Chen, J.L. Anderson, D.S. Sigman, C.S. Foote, Y. Rubin, Tetrahedron 52 (1996) 5179–5189.
[31] Y.N. Yamakoshi, T. Yagami, S. Sueyoshi, N. Miyata, J. Org. Chem. 61 (1996) 7236–7237.
[32] K. Kobayashi, S. Tagawa, J. Am. Chem. Soc. 125 (2003) 10213–10218.
[33] L.P. Candeias, S. Steenken, J. Am. Chem. Soc. 111 (1989) 1094–1099.
[34] A preliminary report on direct observation of radical cations of adenine, cytosine, and thymine radical cation has appeared:
K. Ohkubo, K. Yukimoto, S. Fukuzumi, Chem. Commun. (2006) 2504–2506.
[35] The energies of the 0–0 transition (E_{00}) between the S_1 and the S_0 state were determined by averaging the energies of the corresponding (0,0) peaks in the fluorescence and the absorption bands. The values of E_{00} were obtained as 2.77 eV ($AcrH^+$), 2.72 eV (Acr^iPr^+), and 2.69 eV ($AcrPh^+$), respectively.
[36] The one-electron reduction potentials (E_{red}) of $AcrR^+$ in the absence and the presence of DNA were reported previously, see Ref. [18].
[37] Concentration of DNA per nucleotides of 6600 mol dm^{-3} cm^{-1} at 260 nm. See:
M.E. Reichmann, S.A. Rice, C.A. Thomas, P. Doty, J. Am. Chem. Soc. 76 (1954) 3047–3053.
[38] (a) M. Fujita, A. Ishida, S. Takamuku, S. Fukuzumi, J. Am. Chem. Soc. 118 (1996) 8566–8574;
(b) K. Suga, K. Ohkubo, S. Fukuzumi, J. Phys. Chem. A 107 (2003) 4339–4346.
[39] (a) A.T. Poulos, G.S. Hammond, M.E. Burton, Photochem. Photobiol. 34 (1981) 169–175;
(b) K.S. Peters, E. Pang, J. Rudzki, J. Am. Chem. Soc. 104 (1982) 5535–5537.
[40] K. Ohkubo, K. Suga, S. Fukuzumi, Chem. Commun. (2006) 2018–2020.
[41] It was claimed that the triplet excited state of the acridinium ion moiety ($^3Acr^+$*–Mes) might be formed rather than the electron-transfer state ($Acr^•$–$Mes^{•+}$), see Ref. [42]. However, it has been shown that the observed triplet excited state results from acridine contained as an impurity in the preparation of Acr^+–Mes, see Ref. [43].
[42] (a) A.C. Benniston, A. Harriman, P. Li, J.P. Rostron, J.W. Verhoeven, Chem. Commun. (2005) 2701–2703;
(b) A.C. Benniston, A. Harriman, P. Li, J.P. Rostron, H.J. van Ramesdonk, M.M. Groeneveld, H. Zhang, J.W. Verhoeven, J. Am. Chem. Soc. 127 (2005) 16054–21664.
[43] K. Ohkubo, H. Kotani, S. Fukuzumi, Chem. Commun. (2005) 4520.
[44] There has been reported no interaction between 9-*o*-tolyl-10-methylacridinium ion, see Ref. [19].
[45] The bleaching absorption band around 450–490 nm comes from the absorption band of the $Mes^{•+}$ moiety (λ_{max} = 480 nm);
S.M. Hubig, J.K. Kochi, J. Am. Chem. Soc. 122 (2000) 8279–8288.
[46] K. Ohkubo, K. Suga, K. Morikawa, S. Fukuzumi, J. Am. Chem. Soc. 125 (2003) 12850–12859.
[47] The $AcrR^•$ radical (R = H) decays via the dimerization reaction to afford the dimer $(AcrH)_2$; see:
S. Fukuzumi, T. Kitano, K. Mochida, J. Am. Chem. Soc. 112 (1990) 3246–3247.
[48] No electron transfer occurred from CT DNA to the singlet excited state of acridinium moiety of Acr^+–Mes because the rate of electron transfer is slower than intramolecular electron transfer from mesitylene moiety to acridinium ion moiety.
[49] Suppression of DNA cleavage by intercalation of chromophore in DNA has been also reported in another system, see Ref. [17].