Direct detection of nucleotide radical cations produced by electron-transfer oxidation of DNA bases with electron-transfer state of 9-mesityl-10-methylacridinium ion and resulting efficient DNA cleavage without oxygen†

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Photoinduced electron transfer of DNA as well as DNA bases with 9-mesityl-10-methylacridinium ion results in formation of all types of DNA base radical cations, which have been detected as the transient absorption spectra measurements, leading to efficient DNA cleavage in the absence of O_2.

Photoinduced DNA damage has attracted considerable interest because of the biological significance of DNA damage and repair.[1–7] The DNA cleavage results from the electron-transfer oxidation of DNA bases, followed by hole transfer to the guanine part *in vivo*, which is known to be most readily oxidized among the four DNA bases. Extensive efforts have so far been made to detect guanine radical cation.[8,9] However, so far there has been no report on direct observation of radical cations of the other DNA bases, *i.e.*, adenine, cytosine, and thymine. On the other hand, studies on DNA cleavage have been focused on *intra*molecular electron transfer (ET) using the photosensitizer-modified or intercalated DNA molecules.[10–14] In contrast, the oxidative DNA cleavage by *inter*molecular photoinduced ET oxidation of DNA through a collisional process has yet to be scrutinized, because the short lifetimes of photosensitizers have precluded the oxidative DNA damage at low concentrations, which are required for the study of DNA cleavage.

We have recently found that the photoexcitation of a donor–acceptor dyad, 9-mesityl-10-methylacridinium ion (Acr^+–Mes), results in formation of the ET state ($Acr^{\cdot}$–$Mes^{\cdot+}$) by intramolecular ET from the mesitylene moiety to the acridinium ion moiety, which has an extremely long lifetime (*e.g.*, 2 h at 203 K).[15] $Acr^{\cdot}$–$Mes^{\cdot+}$ can act as a strong oxidant (E_{red} = 1.88 V *vs* SCE),[15] being capable of oxidizing DNA bases, as well as a reductant (E_{ox} = −0.49 V *vs* SCE),[15] which can reduce O_2.

We report herein the *inter*molecular photoinduced ET oxidation of DNA bases with Acr^+–Mes and the first successful detection of the transient absorption spectra of the radical cations of all types of DNA bases, GMP (guanosine 5′-monophosphate), AMP (adenosine 5′-monophosphate), CMP (cytidine 5′-monophosphate), and TMP (thymidine 5′-monophosphate), by nanosecond laser flash photolysis measurements. It should be noted that there is no intercalation of a bulky Acr^+–Mes with double-stranded DNA because the dihedral angle between Acr^+ and Mes moieties of Acr^+–Mes is approximately perpendicular.[15] We have also examined efficient DNA cleavage by *inter*molecular photoinduced ET oxidation of DNA with $Acr^{\cdot}$–$Mes^{\cdot+}$ in the absence and presence of oxygen. To our surprise, the DNA cleavage *in the absence of oxygen* was much more efficient than that in the presence of O_2.

Nanosecond laser excitation at 355 nm of a deaerated buffer solution of Acr^+–Mes results in formation of the long-lived ET state ($Acr^{\cdot}$–$Mes^{\cdot+}$) *via* photoinduced ET from the Mes moiety to the singlet excited state of the Acr^+ moiety at different pH (open circles and open rectangles in Fig. 1a).[15] Since the one-electron reduction potential of $Acr^{\cdot}$–$Mes^{\cdot+}$ (E_{red} = 1.88 V *vs* SCE in acetonitrile)[15] is much more positive than the one-electron oxidation potential of GMP (E_{ox} = 1.07 V *vs* SCE in an aqueous solution),[16] electron transfer from GMP to the $Mes^{\cdot+}$ moiety in $Acr^{\cdot}$–$Mes^{\cdot+}$ may be energetically feasible.[17] Thus, 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 ($GMP^{\cdot+}$: λ_{max} = 510 nm)[8,9] as shown in Fig. 1a (closed rectangles). At pH 7.0, a transient absorption at the long wavelength region at *ca.* 650 nm appears because of the deprotonation of $GMP^{\cdot+}$ (closed circles in Fig. 1a).[8,9] The difference spectra in Fig. 1b, obtained by subtracting the spectra in the absence of GMP from those in the presence of GMP at pH 2.0 and 7.0, correspond to those between $GMP^{\cdot+}$ (positive absorption) and the $Mes^{\cdot+}$ moiety (negative absorption), because the spectra in the absence and the presence of GMP are those of $Acr^{\cdot}$–$Mes^{\cdot+}$ and those of $GMP^{\cdot+}$ and $Acr^{\cdot}$–Mes, respectively.[18] The formation rate of $GMP^{\cdot+}$ 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 (see Fig. S1 in the electronic supplementary information†). The second-order rate constant (k_{et}) of electron transfer from GMP to the $Mes^{\cdot+}$ moiety of $Acr^{\cdot}$–$Mes^{\cdot+}$ is determined as 4.3×10^7 dm^3 mol^{-1} s^{-1} in the buffer solution at 298 K.[19,20] The k_{et} value of electron transfer from GMP to the $Mes^{\cdot+}$ moiety of $Acr^{\cdot}$–$Mes^{\cdot+}$ is 2.7×10^8 dm^3 mol^{-1} s^{-1} at pH 7.0, which is much larger than the value (2.0×10^7 dm^3 mol^{-1} s^{-1}) at pH 2.0, but still smaller than the diffusion limit. The larger k_{et} value at the higher pH indicates the involvement of deprotonation associated with electron transfer (proton-coupled electron transfer).[17] In such a case, the electron transfer at pH 2.0 may not be

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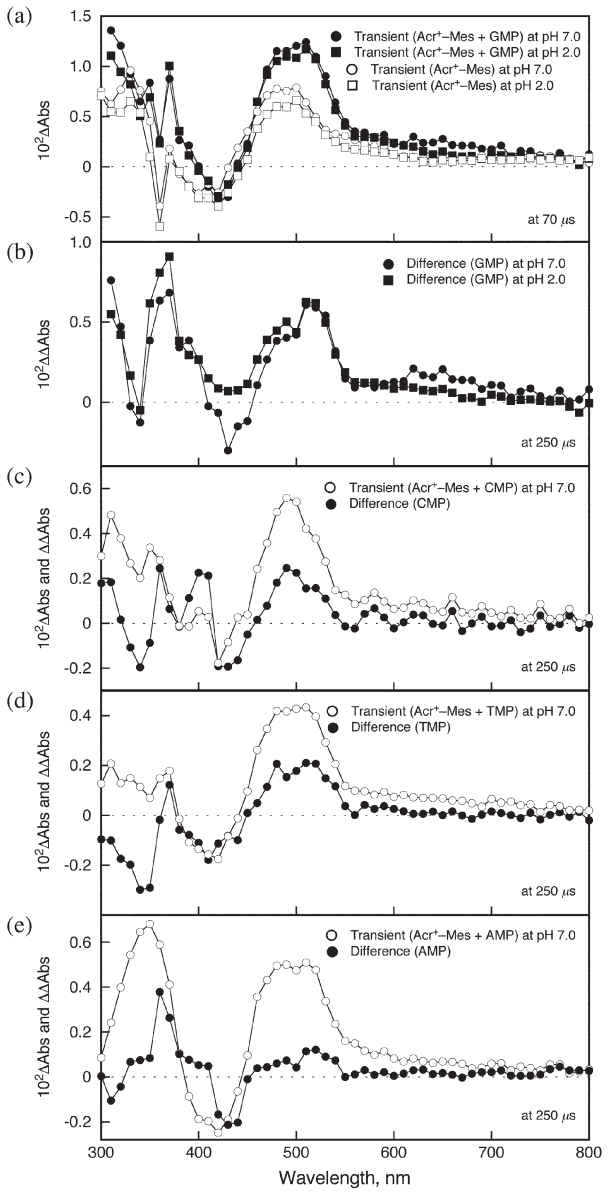


Fig. 1 (a) Transient absorption spectra of Acr^+–Mes in the presence and absence of GMP (1.0×10^{-2} mol dm^{-3}) at pH 2.0 and 7.0. (b) Difference transient absorption spectra of $GMP^{\bullet+}$ (pH 2.0) and $(GMP–H)^{\bullet}$ (pH 7.0), obtained by subtracting the spectra in the absence of GMP from those in the presence of GMP at pH 2.0 and 7.0, respectively. (c) Transient absorption spectrum of Acr^+–Mes in the presence of CMP (7.0×10^{-2} mol dm^{-3}) and the difference spectrum of $CMP^{\bullet+}$ at pH 7.0. (d) Transient absorption spectrum of Acr^+–Mes in the presence of TMP (1.0×10^{-1} mol dm^{-3}) and the difference spectrum of $TMP^{\bullet+}$ at pH 7.0. (e) Transient absorption spectrum of Acr^+–Mes in the presence of AMP (1.0×10^{-1} mol dm^{-3}) and the difference spectrum of $AMP^{\bullet+}$ at pH 7.0. All transient absorption spectra were measured at 70 or 250 μs after laser excitation.

largely endergonic as expected from the reduction potential of the $Mes^{\bullet+}$ moiety of $Acr^{\bullet}$–$Mes^{\bullet+}$ (E_{red} = 1.88 V *vs* SCE) in benzonitrile, because the E_{red} value may be less positive in an aqueous solution as compared with that in an aprotic solvent (benzonitrile).

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. 1c, 1d, and 1e).[18,20] 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 the DNA bases. The k_{et} values of electron transfer at pH 7.0 from CMP, TMP and AMP are also determined as 2.2×10^5, 1.2×10^5 and 7.6×10^5 dm^3 mol^{-1} s^{-1}, respectively (see Fig. S2 in the ESI†).[19] The rate of ET oxidation of GMP is two orders of magnitude faster than those of other nucleobases.

Calf thymus DNA was also efficiently oxidized by the ET state of Acr^+–Mes. Transient absorption spectra of oxidized DNA were similar to those of DNA base radical cations in Fig. 1 (see Fig. S3 in the ESI†). The k_{et} value of the ET oxidation of DNA was determined as 4.8×10^7 dm^3 mol^{-1} s^{-1} (see Fig. S4 in the ESI†),[18] which is even larger than the k_{et} value of GMP.[21]

We also examined the DNA-cleavage activity of Acr^+–Mes in the presence of O_2 using the widely used assay with the double-stranded plasmid DNA, pBR 322, since $O_2^{\bullet-}$ is formed in the ET reaction from the $Acr^{\bullet}$ moiety (E_{ox} = −0.49 V *vs* SCE)[15,22] to oxygen (E_{red} = −0.40 V *vs* SCE).[23,24] The agalose gel electrophoresis was performed after 5 min photoirradiation of pBR 322 with the monochromatized light (λ = 360 nm) in the presence of Acr^+–Mes in comparison with those in the presence of 9-substituted acridinium ions without an electron donor moiety ($AcrR^+$, R = H, ^{i}Pr and Ph) as shown in Fig. 2a.

The reactivity of DNA cleavage increases in the following order: $AcrPh^+ < AcrH^+$, $Acr^iPr^+ \ll Acr^+$–Mes. The low reactivity of $AcrPh^+$ results from the short fluorescence lifetime (1.3 ns) of $^1AcrPh^{+*}$ (* denotes the excited state) as compared with those of $^1Acr^iPr^{+*}$ (26 ns) and $^1AcrH^{+*}$ (31 ns).[25] The highest reactivity has been achieved with Acr^+–Mes because of the extremely long-lived ET state.[15]

To our surprise, it has been found that the DNA cleavage activity with Acr^+–Mes in the absence of O_2 is much higher than that in the presence of O_2 at pH 5.0 and 7.0 as shown in Fig. 2b. This indicates that O_2 acts as an apparent inhibitor for the DNA cleavage. When DNA is oxidized by the $Mes^{\bullet+}$ moiety of $Acr^{\bullet}$–$Mes^{\bullet+}$, O_2 is reduced by the $Acr^{\bullet}$ moiety to produce $O_2^{\bullet-}$.[26,27]

The retarding effect of O_2 may result from the more efficient back ET from $O_2^{\bullet-}$ to DNA radical cation as compared to that from the $Acr^{\bullet}$ moiety to DNA radical cation before oxidizing DNA, as shown in Scheme 1.[28] Although all DNA bases can be oxidized by the $Mes^{\bullet+}$ moiety of $Acr^{\bullet}$–$Mes^{\bullet+}$ (Fig. 1), the largest k_{et} value of the ET oxidation of GMP together with the lowest oxidation potential of GMP among DNA bases (*vide supra*) indicate that guanine is eventually oxidized in ET from DNA to the $Mes^{\bullet+}$ moiety of $Acr^{\bullet}$–$Mes^{\bullet+}$, leading to an efficient DNA cleavage.[29,30] The higher DNA cleavage activity at pH 5.0, as compared with that at pH 7.0 in Fig. 2b suggests that the guanine radical cation has a higher reactivity for the DNA cleavage than the deprotonated radical (Scheme 1) judging from the pK_a value of the guanine radical cation (pK_a = 3.9).[9]

In conclusion, the transient absorption spectra of four nucleotide radical cations have been successfully detected in the ET oxidation of the corresponding DNA bases with the $Mes^{\bullet+}$ moiety of the long-lived ET state ($Acr^{\bullet}$–$Mes^{\bullet+}$), which is produced upon photoexcitation of Acr^+–Mes. DNA is also efficiently oxidized by the $Mes^{\bullet+}$ moiety of $Acr^{\bullet}$–$Mes^{\bullet+}$, leading to efficient DNA cleavage *in the absence of* O_2, as compared to acridinium ions without a donor moiety.

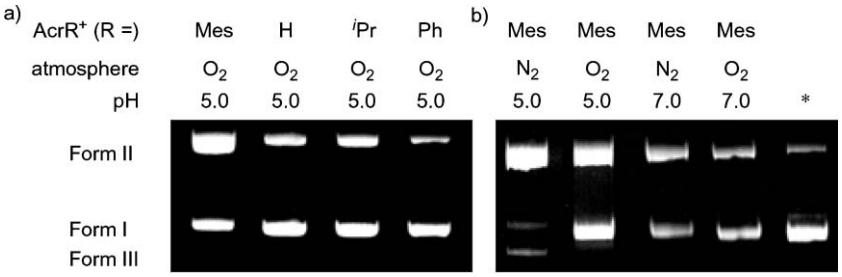
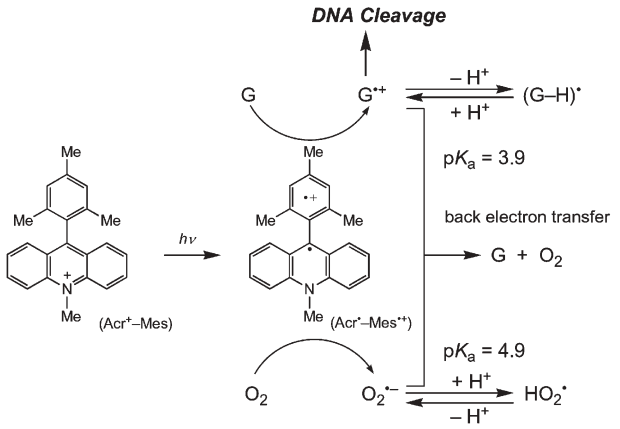


Fig. 2 Agarose gel electrophoresis of photoinduced cleavage of supercoiled pBR322 DNA (0.051 μg μL^{-1}) with (a) various 9-substituted acridinium ions ($AcrR^+$: 1.0 × 10^{-4} mol dm^{-3}) in an O_2-saturated buffer solution (10 mmol dm^{-3} CH_3COOH/KOH, pH 5.0) after 5 min and (b) Acr^+–Mes (1.0 × 10^{-4} mol dm^{-3}) in an N_2– or O_2-saturated buffer solution after 4 min photoirradiation of monochromatized light (λ = 360 nm). The asterisk denotes the control experiment: pBR322 DNA in the presence of Acr^+–Mes before photoirradiation.



Scheme 1

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