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Reductive DNA Cleavage Induced by UVA Photoirradiation of NADH without Oxygen

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Lower-energy UVA photons (320−400 nm) as compared with UVC (190−280 nm) and UVB (280−320 nm) constitute the large majority of terrestrial UV radiation. Although UVA photons are less cytotoxic than UVB light, the potential deleterious effect of UVA has emerged as a source of serious concern for public health because of the recent widespread use of efficient UVB-blocking sunscreens, the popularity of UVA tanning beds, and prolonged periods of sunbathing.[1−3] In the UVB range, direct light absorption by DNA results mainly in dimerization reactions between adjacent pyrimidine bases.[1−3] In contrast, UVA radiation is hardly absorbed by DNA, but rather excites endogenous chromophores, leading to DNA damage.[4] At present, the endogenous chromophores responsible for premutagenic DNA lesions induced by UVA have not been identified. On the other hand, dihydronicotinamide coenzyme (NADH), which plays a key role in a number of biological redox processes including the respiratory chain,[5] absorbs UVA light effectively. However, thus far there has been no report on reductive cleavage of DNA using NADH as a reductant.

We report herein that UVA irradiation of an aqueous DNA solution containing NADH under N_2 results in effective cleavage of supercoiled plasmid DNA. It is shown that the photoinduced DNA cleavage results from photoionization of NADH as well as photoinduced electron transfer from NADH to DNA without oxygen.

The photoinduced DNA-cleavage activity by NADH was examined under N_2 by UVA irradiation using the widely used assay with pBR322 supercoiled DNA by agarose gel electrophoresis.[6] The active oxygen species is known to be formed under UVA irradiation of NADH in the presence of molecular oxygen.[7] UVA light irradiation of an aqueous DNA solution containing NADH in the presence of O_2 also results in cleavage of plasmid DNA (supercoiled form I) to afford the nicked form II under air.[8] To our surprise, however, much more effective photocleavage of plasmid DNA by NADH was observed under N_2 without oxygen as shown in Figure 1.[9]

An aqueous buffer solution (20 μL) of deaerated 10 mM CH_3-COOH/KOH (pH 5.0) containing DNA (4.8 ng μL^{-1}, 7.4×10^{-6} M)[10] and NADH (1.9×10^{-2} M) in a micro test tube was excited by an Nd:YAG laser at $\lambda = 355$ nm (12 mJ/pulse) under N_2. Figure 2a shows the photoinduced DNA cleavage without O_2 with various laser intensities. No photoinduced DNA cleavage by laser irradiation was observed under the deaerated conditions in the absence of NADH. Figure 2b shows a plot of the ratio of cleaved DNA as a function of the laser intensity. The numbers of photons per pulse were determined by the transient absorption due to the triplet−triplet absorption of C_{60} ($\lambda_{max} = 750$ nm, $\epsilon_{max} = 1.8 \times 10^4\ M^{-1}\ cm^{-1}$)[11] in benzene. It is found that the ratio of cleaved DNA is proportional to the square of the laser intensity (Figure 2c). This indicates that the photocleavage of DNA by NADH is a two-photon process.

The photoionization of NADH by laser irradiation is reported to be a two-photon reaction process expressed by eq 1.[12]

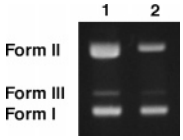

Figure 1. Agarose gel electrophoresis of photoinduced cleavage of supercoiled pBR322 DNA (7.4×10^{-6} M) with NADH (1.9×10^{-2} M) in deaerated 10 mM CH_3COOH/KOH (pH 5.5) at 298 K after 25 min photoirradiation of monochromatized light ($\lambda = 340$ nm) with a xenon lamp. (Lane 1) Under N_2. (Lane 2) Under air.

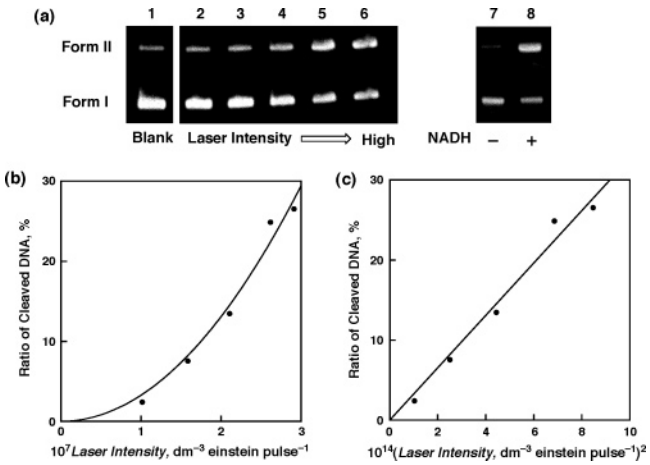

Figure 2. (a) Agarose gel electrophoresis of photoinduced cleavage of supercoiled pBR322 DNA (7.4×10^{-6} M) with NADH (1.9×10^{-2} M) in deaerated 10 mM CH_3COOH/KOH (pH 5.0) at 298 K after irradiation of 32 laser pulses with various laser intensities. (Lane 1) No irradiation. (Lanes 2−6) Photoirradiation with various laser intensities. (Lane 7) Irradiation in the absence of NADH. (Lane 8) Irradiation in the presence of NADH. The irradiation conditions at lanes 7 and 8 are the same. (b) Ratio of cleaved DNA by laser irradiation of NADH plotted against the laser intensity. (c) Ratio of cleaved DNA by laser irradiation of NADH plotted against the square of the laser intensity.

$$\mathrm{NADH} \xrightarrow{h\nu} {}^1\mathrm{NADH}^* \xrightarrow{h\nu} \mathrm{NADH}^{\bullet+} + \mathrm{e_{aq}}^- \qquad (1)$$

The amount of hydrated electron (e_{aq}^-) generated by the photoionization of NADH is proportional to the square of the laser intensity.[12] Thus, DNA cleavage may be caused by e_{aq}^- generated by the photoionization of NADH. The reaction of e_{aq}^- with DNA was examined by the laser flash photolysis experiments (vide infra).

The transient absorption spectra of an aqueous buffer solution (7 mM KH_2PO_4/NaOH; pH 7.0) containing NADH (1.7×10^{-4} M) were observed upon the laser excitation. The strong absorption in the near-infrared region ($\lambda_{max} = 715$ nm) is attributed to e_{aq}^-.[12] In the presence of nucleotides [thymidine 5′-monophosphate (TMP), cytidine 5′-monophosphate (CMP), adenosine 5′-monophosphate (AMP), and guanosine 5′-monophosphate (GMP)], the decay rates of e_{aq}^- become much faster than those in the absence of nucleotide (see Supporting Information S2). The decay time profile in the

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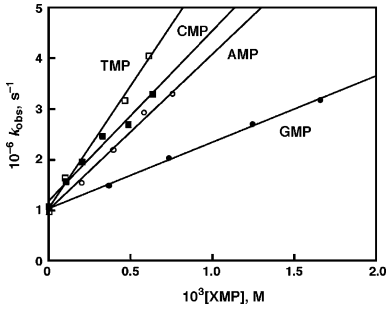

Figure 3. Plots of the pseudo-first-order rate constant (k_{obs}) vs concentration of nucleotides, [XMP]: [TMP], [CMP], [AMP], and [GMP] for the reactions of e_{aq}^- with nucleotides at 298 K.

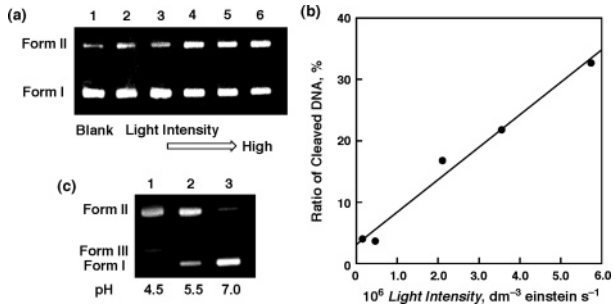

Figure 4. (a) Agarose gel electrophoresis of photoinduced cleavage of supercoiled pBR322 DNA (7.4×10^{-6} M) with NADH (1.9×10^{-2} M) in deaerated 10 mM CH_3COOH/KOH (pH 5.0) at 298 K after 25 min photoirradiation with monochromatized light ($\lambda = 340$ nm) using a xenon lamp. (Lane 1) No irradiation. (Lanes 2−6) Photoirradiation with various light intensities. (b) Ratio of cleaved DNA plotted against the light intensity. (c) pH dependence of photocleavage ratio in a deaerated aqueous buffer solution at 298 K under N_2. (Lane 1) 10 mM CH_3COOH/KOH (pH 4.5). (Lane 2) 10 mM CH_3COOH/KOH (pH 5.5). (Lane 3) 10 mM KH_2PO_4/NaOH (pH 7.0).

presence of nucleotides obeys pseudo-first-order kinetics. The pseudo-first-order rate constants (k_{obs}) increase linearly with increasing concentrations of nucleotides to exhibit first-order dependence (Figure 3). The rate constants (k_{et}) of electron transfer from e_{aq}^- (which is generated by photoionization of NADH, to TMP, CMP, AMP, and GMP at pH 7.0) are determined from the slopes of linear plots of k_{obs} vs concentrations of nucleotides as 4.8×10^9, 3.4×10^9, 3.1×10^9, 1.3×10^9 M^{-1} s^{-1}, respectively. The k_{et} values largely agree with those reported by using pulse radiolysis.[13] The reaction of e_{aq}^- with DNA has been reported to trigger DNA cleavage.[14] In the presence of O_2, e_{aq}^- is quenched to produce $O_2^{\bullet -}$ which also results in DNA cleavage, but the reactivity is significantly smaller as compared with that of e_{aq}^-.

DNA is also cleaved by one-photon excitation of NADH under N_2 with a xenon lamp. An aqueous buffer solution (20 μL) of deaerated 10 mM CH_3COOH/KOH (pH 5.0) containing DNA (7.4×10^{-6} M) and NADH (1.9×10^{-2} M) in a micro test tube was excited by monochromatized light ($\lambda = 340$ nm) from a xenon lamp at 298 K. Figure 4a shows that NADH cleaves DNA (supercoiled form I) to afford the nicked form II under N_2 with various light intensities. Figure 4b represents a plot of the ratio of cleaved DNA as a function of the light intensity. A standard actinometer, potassium ferroxalate, was used for the determination of the numbers of photons per second. In contrast to the case of the laser excitation (Figure 2b), the ratio of cleaved DNA is proportional to the laser intensity. Since the singlet excited state of NADH has scarcely been quenched by DNA due to the short fluorescence lifetime (0.40 ns),[15] the DNA cleavage may be induced by electron transfer from the triplet excited state of NADH (^{3}NADH*) to DNA, because the one-electron oxidation potential of ^{3}NADH* ($E_{ox}^* =$ -2.2 V vs SCE)[16−18] is low enough to render the electron transfer thermodynamically feasible.[19,20]

We have also examined DNA photocleavage by NADH at various pH conditions under N_2. The more efficient DNA cleavage is observed under lower pH conditions (Figure 4c). Thus, protonation of nucleobases (B) plays an important role in the DNA cleavage. The radical anions of nucleobases ($B^{\bullet -}$) are converted to the neutral radical $B(H)^\bullet$ by protonation.[19,21] The H-abstraction from the proximal sugar moiety of DNA by $B(H)^\bullet$ may result in DNA cleavage.[22,23]

In conclusion, the UVA photoirradiation of NADH induces much more efficient DNA cleavage under anaerobic conditions than aerobic conditions via photoionization of NADH and the subsequent reaction of e_{aq}^- with DNA as well as electron transfer from ^{3}NADH* to DNA. Thus, NADH is a potential endogeneous chromophore acting as a photoreductant of DNA, leading to the DNA cleavage.

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Supporting Information Available: DNA cleavage by an NADH analogue (S1) and kinetic data for the quenching of e_{aq}^- by nucleotides (S2). This material is available free of charge via the Internet at http://pubs.acs.org.

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