Base-Selective Oxidation and Cleavage of DNA by Photochemical Cosensitized Electron Transfer†

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ABSTRACT: A photochemical mechanism for single-strand cleavage of DNA is proposed in which a photoexcited intercalator transfers an electron to an externally bound cosensitizer. Once formed, the oxidized intercalator oxidizes an adjacent base, creating a charge-separated complex from which reactions leading to cleavage of the sugar–phosphate backbone occur in competition with back electron transfer. Using ethidium bromide (EB) as the intercalator and methyl viologen (MV) as the externally bound cosensitizer, a 10-fold enhancement in the rate of single-strand break formation was found in pBR322 DNA over that for EB alone using 488-nm excitation. The rate of cleavage correlated with the amount of MV bound to DNA. In accord with the expected redox properties of the one-electron-oxidized EB and the DNA bases, cleavage occurs selectively at guanines. Although the reaction proceeds in nitrogen-purged solutions, the rate of cleavage in air-saturated solutions was enhanced 2-fold. Treatment of irradiated samples with alkali leads to a 2-fold increase in the yield of single-strand breaks. These results support a mechanism in which cleavage occurs by selective oxidation of guanines in DNA, initiated by photochemical cosensitized electron transfer from intercalated EB to externally bound MV, and may provide a basis for the development of light-activated base-selective DNA cleaving agents.

Designing molecules that cause site-selective or sequence-specific modifications of DNA may have a significant impact on the analysis of genomic DNA because such compounds may offer a clean and efficient way of cutting DNA at sites that are not recognized by conventional restriction enzymes. A number of compounds have been found to have a remarkable ability to recognize and bind to specific sequences or structural domains in DNA (Dervan, 1986; Bailly et al., 1990; Friedman et al., 1991; Kirshenbaum et al., 1988), and many of these compounds cause DNA cleavage with the addition of other agents (Taylor et al., 1984; Mei & Barton, 1988). Because many compounds photosensitize formation of single-strand breaks in DNA (Kochevar & Dunn, 1990), recent efforts have aimed at directing these reagents to specific sequences or domains in DNA (Perrouault et al., 1990; Saito et al., 1989; Henricksen et al., 1991). Such a "photonuclease" would combine the ability to recognize a specific DNA sequence with the ability to cause efficient photochemical strand cleavage at that site. We are exploring some of the elements that may be required in the design of a photosensitizer which is highly efficient and site-selective in the formation of single-strand breaks in DNA.

DNA strand breaks can result from initial damage to either the bases or sugar–phosphate backbone. Studies of the effect of high-intensity UV laser radiation or γ radiolysis have led to the proposal that DNA strand breaks result from initial formation of oxidized bases, particularly guanine (Sevilla et al., 1979; Croke et al., 1988; Opitz & Schulte-Frohlinde, 1987; Gorner et al., 1992). Photosensitized DNA cleavage may also be initiated by oxidation of bases since a mechanism involving oxidation of guanine by electron transfer to methylene blue would explain the guanine-selective cleavage observed with this photosensitizer (Blau et al., 1987; OhUigin et al., 1987).

In order to develop photosensitized DNA cleavage by an electron-transfer mechanism into a practical tool, it is necessary to overcome the inefficiency associated with this process. For example, the photosensitization of strand breaks in DNA by methylene blue has a maximum quantum yield of only 3×10^{-7} (Blau et al., 1987). The inefficiency may be due to efficient back electron transfer between the intercalated methylene blue and the adjacent base. Figure 1 shows two mechanisms for photosensitization involving a cosensitizer that would inhibit back electron transfer and possibly increase the yield of strand breaks. Path A involves the transfer of an electron from an adjacent base to an intercalated photosensitizer in its excited state (step A-1) which then transfers the electron to an externally bound cosensitizer before back electron transfer can occur (step A-2). Path B involves the transfer of the electron from the excited intercalator to the external cosensitizer (step B-1) followed by oxidation of the adjacent base by the oxidized intercalator (step B-2). Both processes produce a metastable complex in which back electron transfer is inhibited by spatial separation of charges and from which the oxidized base could subsequently develop into a single-strand break.

In order to test the validity of this mechanism, we have investigated the ability of the cosensitization pair, ethidium bromide (EB) and methyl viologen (MV), to induce single-strand breaks in DNA. Recent studies have shown that intercalated EB transfers an electron to externally bound MV and that a fraction of the reduced MV dissociates from DNA before back electron transfer (Atherton & Beaumont, 1987; Frohmerz & Reiger, 1986). Thus, competition with back electron transfer appears possible and oxidation of the adjacent base by oxidized EB may occur. This corresponds to path B in Figure 1. Since base oxidation leads to the formation of single-strand breaks in DNA and guanine is the most easily

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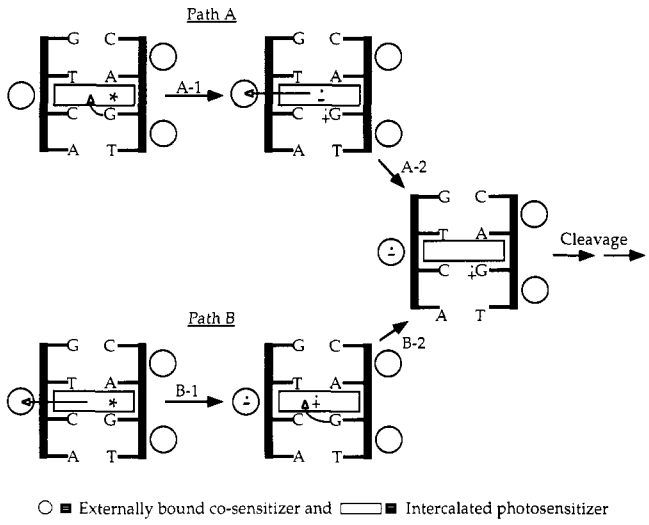

FIGURE 1: Two pathways for light-induced cleavage of DNA involving electron transfer and a pair of cosensitizers. In path A, the photoexcited intercalated sensitizer accepts an electron from guanine (step A-1) and subsequently an electron is transferred from the reduced photosensitizer to an externally bound cosensitizer (step A-2). In path B, the photoexcited intercalated photosensitizer transfers an electron to the externally bound cosensitizer (step B-1) and subsequently accepts an electron from an adjacent guanine (step B-2).

oxidizable base (Kittler et al., 1980), cosensitization may lead to selective formation of DNA strand breaks at guanines.

MATERIALS AND METHODS

Materials. Ethidium bromide and methyl viologen were obtained from Sigma Chemical Co. and were used as received. pBR322 DNA (90%–95% supercoiled) was obtained from Boehringer-Mannheim Biochemical. All solutions used for irradiations were prepared in distilled/deionized water which was filtered through a 22-μm cellulose acetate filter. For each experiment, sterile stock solutions were prepared by diluting the DNA with previously prepared stock solutions of EB and MV and water. DNA concentrations were determined by using the average value of 6600 M^{-1} cm^{-1} for the extinction coefficient of a single nucleotide at 260 nm.

For restriction digestion and end-labeling of the DNA, the enzymes *Eco*R1 (Boehringer-Mannheim), *Cla*1 (New England BioLabs), and DNA polymerase I (Klenow fragment; Boehringer-Mannheim) were used. [α-^{32}P]dATP (3000 Ci/mmol) was obtained from New England Nuclear.

For neutral agarose electrophoresis, type II medium EEO agarose (Sigma) was used. All reagents for preparing and running the agarose gels were obtained from Sigma. For denaturing polyacrylamide gel electrophoresis, reagents and buffers from National Diagnostics Corp. were used.

Irradiations. All irradiations used the 488-nm output from an argon ion laser (Coherent) delivered by an optical fiber. A lens was used to adjust the size of the irradiation area. Typically, the laser power was 140–150 mW exciting from the fiber, and the lens was adjusted to provide a circular spot of 22 cm^2. The samples (40–60 μL) were irradiated from above in individual wells of a sterile 96-well flat-bottomed tissue culture plate placed on a black background. For the irradiations carried out in air-saturated or nitrogen-purged solution, the fiber and lens were turned so that the laser output (125 mW) was focused upward onto the bottom of a glass plate (8.6-cm^2 spot size). For nitrogen-purged samples, 0.5-mL vials containing the sample were out-gassed on ice for 3 min with nitrogen via a sterile 16-gauge needle before irradiation.

The quantum yield for single-strand break formation in pBR322 DNA is determined from the number of supercoiled DNA plasmids (sc-DNA) converted to the relaxed, open circular form (oc-DNA) divided by the number of incident 488-nm photons absorbed by the sample at conversion of less than 15%. The number of plasmids converted was calculated from the percent loss of sc-DNA after a given irradiation dose. The number of incident photons absorbed was calculated using a value of 7000 M^{-1} cm^{-1} for the extinction coefficient of EB at 488 nm, the concentration of EB bound to the DNA [calculated using a value of 2×10^6 M^{-1} for the binding constant of EB with DNA (Frohmerz & Reiger, 1986)], and a path length defined by the average height of the sample in the well. The samples were optically thin, typically having an optical density at 488 nm of less than 0.02.

Neutral Agarose Gel Electrophoresis. Agarose gels (1%) were prepared in sterile 50 mM NaCl/1 mM EDTA. Electrophoresis was carried out in a running buffer consisting of 40 mM Tris base, 20 mM sodium acetate, 2 mM EDTA, and 18 mM NaCl, adjusted to pH 8.05.

Samples were prepared for electrophoresis by diluting a 5-μL aliquot (250 ng of DNA) in TE buffer (10 mM Tris·HCl, 1 mM EDTA, pH 7.4) to a final DNA concentration of 8 ng/μL. For alkali-treated samples, 5 μL of sample (250 ng of DNA) was diluted with 15 μL of TE buffer (pH 7.4) and 2.2 μL of 1 N NaOH. The samples were incubated at 37 °C for 30 min and then neutralized with 1.8 μL of 1 N HCl. Samples (40–50 ng of DNA) were loaded in duplicate into wells of the gel. Electrophoresis was carried out using a Bio-Rad Mini-Sub Cell apparatus at 40 V. After electrophoresis was complete (approximately 60 min), the gel was stained with ethidium bromide (1 μg/mL). The stained gels were photographed on Polaroid 667 film using a Foto-UV 300 transilluminator (Fotodyne Inc.) and a Polaroid MPF camera equipped with UV and color correction filters. The exposure was in the linear response range of the film and densitometer. The photographs were scanned in duplicate using a Camag TLC scanner II (Camag Scientific) in the reflectance mode at 550 nm.

Single-strand breaks were quantitated by calculating the fraction of supercoiled pBR322 DNA (F_{sc}) remaining following irradiation. F_{sc} was determined from the average integrated areas of the densitometry peaks corresponding to sc-DNA and oc-DNA according to

$$F_{sc} = \frac{\text{(area sc-DNA)}}{\text{(area sc-DNA)} + [\text{(area oc-DNA)}/(1.66)]} \quad (1)$$

The factor of 1.66 is included to correct for the greater fluorescence obtained from ethidium bromide bound to oc-DNA compared to sc-DNA (Ciulla et al., 1989). For samples treated with alkali, F_{sc} was calculated from the integrated area of the densitometry peak corresponding to sc-DNA after irradiation, divided by that obtained from an unirradiated sample. The data in Figure 2 are plotted according to the following equation, which shows the first-order dependence of F_{sc} on fluence (f in J/cm^2):

$$\ln (F_{sc}) = -kf + \ln (F^\circ_{sc}) \quad (2)$$

where k is the rate at which single-strand breaks are formed per J/cm^2 delivered at 488 nm and F°_{sc} is the fractional amount of sc-DNA in the unirradiated sample.

Polyacrylamide Gel Electrophoresis. Irradiated samples were prepared for polyacrylamide gel electrophoresis by extracting the photosensitizers with 1-butanol saturated with TE buffer (pH 7.4), and the nucleic acids were concentrated

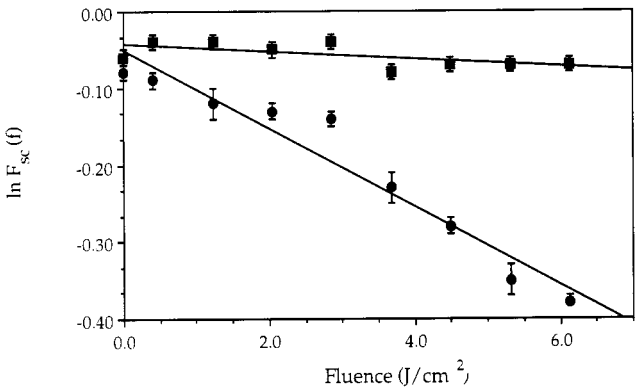

FIGURE 2: Single-strand break formation in pBR322 DNA induced by 488-nm irradiation of solutions containing 150 μM DNA and 20 μM EB (■) or 20 μM EB and 200 μM MV (●). The error bars represent the standard deviation in the average of three separate measurements.

by ethanol precipitation. The samples were then reconstituted to their original concentration in sterile water. A fraction of each sample (containing approximately 0.5 μg of DNA) was then subjected to restriction digestion with 1 unit each of *Eco*R1 and *Cla*1. The restriction fragment was labeled using DNA polymerase I and 10 μCi of [α-^{32}P]dATP. For comparison, unirradiated pBR322, digested and labeled in the same manner, was subjected to chemical sequencing by the Maxam–Gilbert method (Manniatis et al., 1982). Maxam–Gilbert sequencing was carried out using Sigma sequencing kit no. SEQ 1.

The DNA samples, in 10 μL of formamide loading buffer, were incubated at 75 °C for 2 min, and 5 μL was loaded onto a 6% polyacrylamide gel. The electrophoresis was carried out at 1690 V in TBE buffer (0.089 M Tris base and 0.089 M boric acid) for 2 h. After the electrophoresis was complete, the gel was transferred to filter paper, dried, and exposed using X-OMAT AR-5 film from Kodak.

RESULTS AND DISCUSSION

Photosensitized Formation of Single-Strand Breaks in DNA by Ethidium Bromide plus the Cosensitizer Methyl Viologen. Previous studies of the binding of EB and MV to double-stranded (ds-DNA) indicated that EB binds by intercalation while MV appears to bind externally within the grooves of DNA (Fromherz & Rieger, 1986). The binding constant for EB with ds-DNA in the presence of 10^{-4} M MV is 2×10^6 M^{-1} in 1.5 mM cacodylate buffer (pH 6). The binding constant for MV under the same conditions is 1.8×10^5 M^{-1}. The maximum ratio of bound dye to DNA base pair is 0.4 for EB and 1 for MV.

Aqueous solutions of pBR322 DNA (150 μM) were irradiated in the presence of 20 μM EB either with or without 200 μM MV. On the basis of the binding constants approximately 96% of the EB and 37% of the MV are bound at these concentrations. The irradiations were carried out using the 488-nm output of an argon laser (6.8 mW/cm^2). Figure 2 shows the dependence of the fraction of sc-DNA remaining as a function of fluence, plotted according to eq 2. The presence of 200 μM MV during the photosensitization by EB enhanced the rate of formation of strand breaks by a factor of 10 compared to that observed with EB alone (Table I). No loss of sc-DNA was observed when solutions were irradiated with MV alone. From the data in Figure 2, the quantum yield for EB-sensitized cleavage is 5×10^{-8} and that for EB in the presence of MV is 6×10^{-7}.

Table I: Irradiation at 488 nm of Aqueous Solutions of Ethidium Bromide and Methyl Viologen in the Presence of pBR322 DNA

experimental conditions	[EB], μM	[MV], μM	[DNA], μM	slope, cm^2 J^{-1}	change in F_{sc}-DNA, %
air saturated	20	200	150	0.021	
	20	0	150	0.002	
nitrogen purged	20	200	150	0.008	
0.68 mg/mL SOD	20	200	150	0.020	
untreated (4.4 J/cm^2)	20	200	150		11
alkali treated (4.4 J/cm^2)	20	200	150		23
no Na_2SO_4 (6.1 J/cm^2)	20	200	150		13
10 mM Na_2SO_4 (6.1 J/cm^2)	20	200	150		11

This result supports the mechanism shown in Figure 1. Since the singlet excited state of EB is energetically incapable of accepting an electron from DNA bases (Kittler et al., 1980), a cosensitization reaction must occur via path B. Spectroscopic investigations of EB–DNA complexes indicate that the excited singlet state of EB transfers an electron to MV (Atherton & Beaumont, 1987). Upon the addition of MV (200 μM), quenching of the EB–DNA fluorescence in the presence of ds-DNA occurred with an efficiency which was 5 orders of magnitude greater than that in the absence of DNA. Oxidized EB and reduced MV were identified spectroscopically. Fromherz and Reiger (1986) observed a similar enhancement in the fluorescence quenching of EB bound to DNA and the formation of the reduced form of MV. In both cases, the authors propose that fluorescence quenching occurs by electron transfer from the excited singlet state of intercalated EB to externally bound MV.

Binding Requirements for the Cosensitizer. According to the mechanism in Figure 1, the formation of single-strand breaks using MV as a cosensitizer requires the binding of MV and EB to DNA. Thus, the yield of strand breaks should increase with increasing [MV] up to the point that the binding of the cosensitizer reaches saturation. Figure 3A shows that, for a constant amount of light delivered (6.93 J/cm^2 at 488 nm) at 31 μM EB, the fraction of sc-DNA remaining decreases with increasing [MV] and appears to plateau at [MV] greater than 75 μM. Fromherz and Reiger (1986) using somewhat different buffer conditions also found that the quenching of EB–DNA fluorescence increased as the [MV] increased but was less dependent on [MV] when the *free* [MV] reached about 10 μM. Our results suggest that beyond approximately 85 μM MV the binding of MV becomes limiting. A plot of the data from Figure 3A as a function of [MV] bound to DNA, calculated using an association constant of 1.8×10^5 M^{-1}, is linear as shown in Figure 3B. Thus, the photosensitized cleavage appears to require the presence of bound MV as a cosensitizer.

The relationship between the quenching of EB fluorescence by MV and enhanced strand break formation was further examined by evaluating the effect of added salt. Atherton and Beaumont (1987) showed that increasing the concentration of Na_2SO_4 from 1.25 to 10 mM led to a 2-fold decrease in the quenching of EB fluorescence by MV and that the fraction of reduced MV which escaped the DNA helix increased by nearly a factor of 2. These effects were interpreted as showing diminished binding affinity of MV to the external surface of the DNA helix.

The effect of Na_2SO_4 on the photosensitized strand cleavage by EB and MV showed an insignificant change in the amount of DNA cleaved as the [Na_2SO_4] is increased from 0 to 10 mM (Table I). This may simply indicate that the yield of oxidized EB stays approximately constant because of a balancing of two effects, namely, the number of oxidized EB

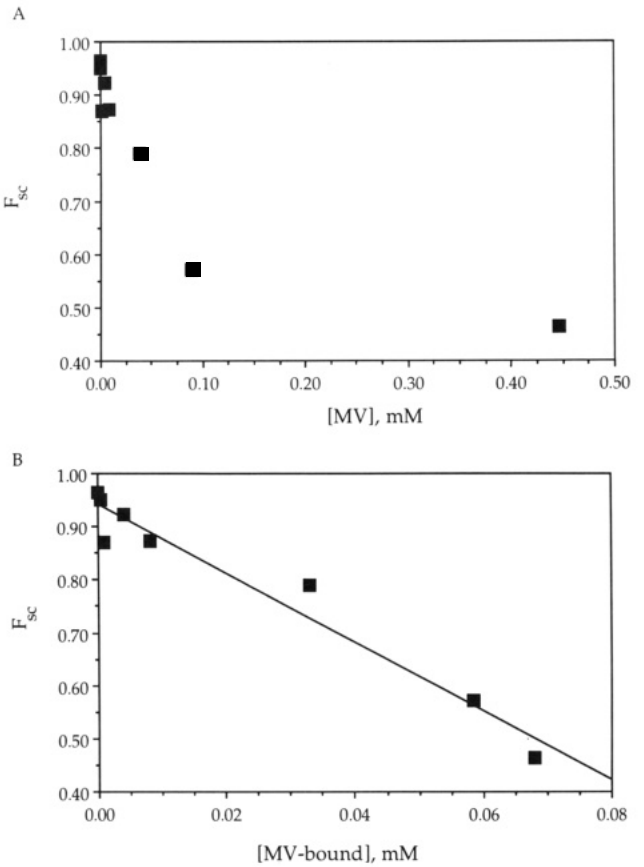

FIGURE 3: Dependence of single-strand break formation in pBR322 DNA on total MV concentration at a constant 488-nm fluence of 6.93 J/cm^2. Panel A: The fraction of sc-DNA remaining was calculated according to eq 1 with each point representing the average of duplicate lanes on an agarose gel. [DNA] = 150 μM; and [EB] = 31.3 μM. Panel B: Data from panel A plotted as a function of the concentration of MV bound externally to DNA using an association constant of 1.8×10^5 M^{-1}.

molecules is lower at high salt due to the inhibition of fluorescence quenching by salt, but the number of oxidized EB molecules undergoing back electron transfer also decreases since more reduced MV molecules escape.

Base Selectivity. In order to examine the base selectivity of cosensitized DNA cleavage, pBR322 DNA was irradiated with EB plus MV and the products were analyzed by high-resolution polyacrylamide gel electrophoresis. Aqueous solutions containing 150 μM DNA, 20 μM EB, and 210 μM MV were irradiated at 488 nm (2.28 and 4.56 J/cm^2; intensity = 7.6 mW/cm^2). Under these conditions, approximately 50% of the sc-DNA was converted to the open-circular form. A fraction of each sample was then subjected to restriction digestion with the enzymes *Eco*R1 and *Cla*1, end labeled, and analyzed on a 6% polyacrylamide gel. For comparison, unirradiated pBR322, digested and labeled in the same manner, was subjected to chemical sequencing by the Maxam–Gilbert method (Maniatis et al., 1982).

Figure 4 shows that EB plus MV photosensitizes the formation of single-strand breaks at selected bases along the polynucleotide chain. The pattern of cleavage resembles most closely that obtained from the Maxam–Gilbert "G" reaction except that the photoproducts show slightly lower mobility. This is similar to the results obtained from methylene blue sensitization of DNA cleavage and 248-nm excimer laser-induced cleavage of ds-DNA (Blau et al., 1987; Croke et al., 1988). For methylene blue photosensitization, treatment of the photoproducts with alkali caused the bands to migrate

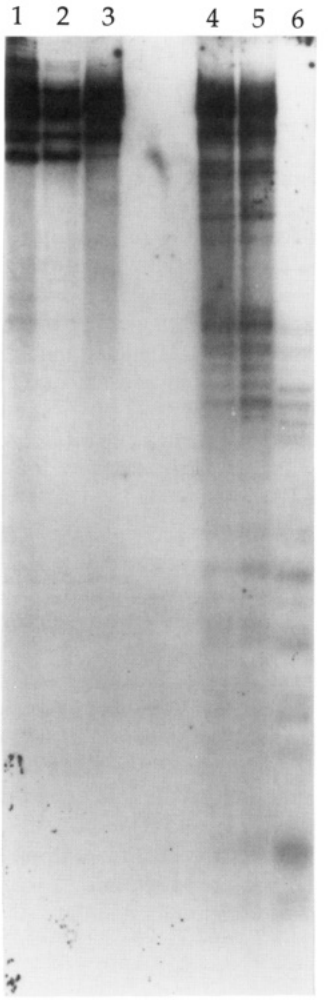

FIGURE 4: Polyacrylamide gel of pBR322 DNA, digested with *Cla*1 and *Eco*R1 and end labeled as described in Materials and Methods. Lane 1: Unirradiated DNA. Lane 2: 150 μM DNA, irradiated with 4.56 J/cm^2 of 488-nm light. Lane 3: 150 μM DNA, 20 μM EB, and 210 μM MV, unirradiated. Lanes 4 and 5: Same as lane 3 but irradiated at 488 nm at fluences of 2.28 and 4.56 J/cm^2, respectively. Lane 6: Unirradiated DNA, cleaved at guanines according to the Maxam–Gilbert method.

with the same mobility as the corresponding "G" products. Identical results were obtained from excimer laser-induced cleavage (Croke et al., 1988). The authors proposed that cleavage is initiated by oxidation of guanine and subsequent rupture of the 3′-sugar–phosphate bond, leaving a modified guanosine residue which can be removed by treatment with alkali. A similar product is expected in our experiment since oxidized guanine is the primary DNA product. Other possibilities for the decreased mobility of the photoproducts have not been ruled out, such as the formation of an adduct between the photosensitizer and the nucleoside at the site of cleavage. Adduct formation has been observed between other photosensitizers and DNA under conditions in which guanine has been photooxidized (Kelly et al., 1990). Overall, our results demonstrate that EB plus MV causes strand breaks selectively at guanine.

These results contrast significantly with those obtained by using EB as a photosensitizer alone. Irradiation of EB in the presence of DNA causes the formation of single-strand breaks almost randomly along the polynucleotide chain (Krishnamurthy et al., 1990). The proposed mechanism involves direct attack of the excited singlet state of EB on the DNA sugar–phosphate backbone. Thus the addition of MV significantly

modulates the photosensitizing mechanism of EB from a nonspecific mechanism to a base-selective cleavage mechanism.

Selectivity at guanine is consistent with the relative oxidation potential of the bases and EB. The oxidation potential for EB is 1.68 V vs NHE (Fromherz & Reiger, 1980). Of the four bases in DNA, guanine has the lowest oxidation potential with a value of 1.53 V vs NHE (Kittler et al., 1980). Thus, the free energy for oxidation of guanine by one-electron-oxidized EB should be –0.15 V, which is more favorable than those with the other bases (–0.05 V for adenine, 0.1 V for thymine, and 0.25 V for cytosine). Oxidation of DNA bases has been shown to lead to single-strand breaks when the oxidized base was produced by 248-nm laser radiation (Opitz & Schulte-Frohlinde, 1987), and 193-nm radiation (Gorner et al., 1992), and by other photosensitizers such as thiopyronine (Berg et al., 1978) and 3-carbethoxypsoralen (Sage et al., 1989).

Effect of Oxygen. Oxygen enhances the efficiency of formation of strand breaks sensitized by EB plus MV. The rate of cleavage increases by a factor of 2 in air-saturated solution compared to nitrogen-purged solutions (Table I). A mechanism involving singlet oxygen can be ruled out since formation of strand breaks increases as a function of the amount of MV bound to DNA. Since MV quenches EB singlet states, fewer EB triplets are formed. If singlet oxygen were responsible for strand breaks, the yield of strand breaks should decrease with increasing [MV]. The reduced form of MV is easily oxidized by oxygen, and therefore, superoxide could be a reactive intermediate. In order to investigate the involvement of superoxide, the photosensitization was carried out in the presence of superoxide dismutase (SOD). As shown in Table I, irradiation of solutions containing 0.68 mg/mL SOD caused no change in the number of strand breaks formed relative to that observed in the absence of the enzyme.

Oxygen was found to be unnecessary for strand break formation induced in single-stranded DNA and poly(A) by 193-nm irradiation (Gorner et al., 1992) and methylene blue photosensitization of double-stranded DNA (Blau et al., 1987). In both cases cleavage is believed to be initiated by the formation of an oxidized base. The mechanism by which the oxidized base converts to a single-strand break may involve direct abstraction of the hydrogen atom at the C-4′position of an adjacent sugar by the neutral base radical which is formed following deprotonation of the base radical cation (Gorner et al., 1992). Once formed, the C-4′ sugar–phosphate radical is believed to undergo decomposition by cleavage of the phosphodiester bond at the 3′ position. Other pathways to strand break formation, however, can become available in the presence of oxygen such as the formation of base or sugar peroxy radicals. For example, the mechanism of Fe^{2+}–bleomycin-mediated cleavage of DNA in the presence of oxygen is postulated to involve the formation of a C-4′ peroxy radical, leading to the release of a base propenal and cleavage of the sugar–phosphate backbone (Rabow et al., 1990). In the case of EB plus MV sensitized cleavage, oxygen could be involved in attack on the oxidized guanine or oxygen could react with the C-4′ sugar radical as in the case of bleomycin. Our results do not allow us to make a definitive statement on the mechanistic role of oxygen in the cosensitized cleavage reaction.

Effect of Alkali. Our results show a 2-fold increase in the fraction of sc-DNA converted to the relaxed form over that obtained without alkali treatment (Table I). In addition, the efficiency of photosensitized cleavage by EB plus MV increased by a factor of 4 when the samples are stored in the dark at room temperature up to but not beyond 80–120 h. The dark reaction occurs even when the samples are stored under nitrogen or when the photosensitizers are extracted from the solution following irradiation. Thus, dark reactions initiated by oxygen or a reactive species derived from the photosensitizers are not involved. The same base selectivity shown in Figure 4 is observed with samples which have been stored in the dark after irradiation. A similar dark reaction is not observed for photosensitization by EB alone (data not shown), which serves as further evidence that EB alone photosensitizes cleavage by a different mechanism. The nature of the DNA lesions that are converted by alkali or heat into strand breaks is still being investigated.

Guanine-specific strand breaks after treatment with hot piperidine were also reported when 3-carbethoxypsoralen was used as a photosensitizer (Sage et al., 1990). Our results indicate that cosensitization by EB and MV initiates the formation of a lesion in DNA at guanines which is unstable and can develop into a strand break slowly in the absence of alkali or more rapidly after alkali treatment. Further investigations are required to elucidate the nature of this lesion.

In summary, our results are consistent with a photochemical cosensitization mechanism for formation of single-strand breaks in double-stranded DNA. This is demonstrated by the enhanced rate of cleavage when both EB and MV are present during the irradiation compared to EB alone and by the dependence of the cleavage on the amount of MV bound to DNA. Site-selective cleavage at guanine is also consistent with this mechanism. These results may serve as a basis for further design and development of a highly efficient, base-selective photonuclease.

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