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Light-Induced DNA Cleavage by Bis-9-acridinyl Viologen Derivative

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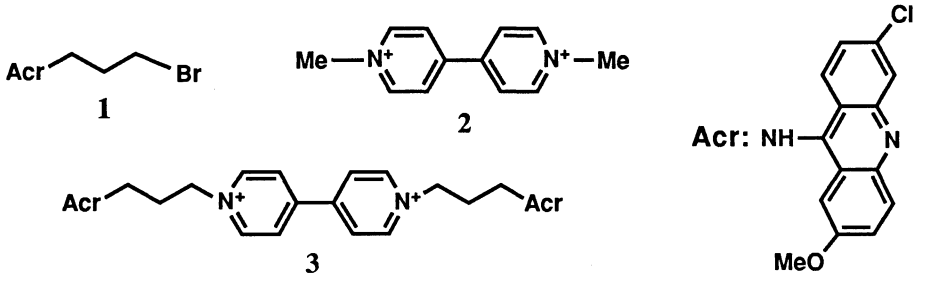
A bis-9-acridinyl derivative containing a viologen linker chain (**3**) forms an intercalation complex with DNA, and was found to cleave the double chain DNA strand effectively under irradiation by visible light, due to the photosensitized intramolecular electron transfer from the acridine to viologen moieties within **3**.

Photochemical DNA cleaving agents are an attractive tool in processing DNA. Many studies are devoted to the design of sequence-specific DNA cleaving agents.[1] On the other hand, relatively nonsequence-specific DNA photocleaving agents, especially those which cleave DNA with lower sequence specificity than DNaseI are useful for DNA photo-footprinting and photosequencing.

Simple intercalating dyes such as Methylene Blue[2] and Acridine Orange[3] have been shown to form an intercalation complex with DNA and photocleave double stranded DNA. However, the conditions required are rather severe (UV and strong light) and the cleavage efficiency is low. In spite of these limitations, intercalator-based photocleaving agents are quite attractive because of their structural and functional simplicity coupled with the possibility of resolving such limitations by an improved molecular design. On the other hand, it is known that a photoexcited ethidium molecule transfers an electron to methyl viologen on DNA matrix.[4] Also it is known that a one electron-reduced viologen reacts with dioxygen to produce superoxide[5] and that the secondary products of its decomposition are capable of cleaving DNA.[6] These considerations lead us to design a new "photonuclease"-type compound based on the combination of a photosensitizing, intercalating dye and an electron mediator (viologen). In the present paper, we report preliminary findings on the DNA photocleaving properties of a novel bis-9-acridinyl derivative containing a viologen linker chain (**3**). The bis-9-acridinyl derivative **3** was synthesized by the reported procedure.[7] Photocleavage of plasmid pBR322 was studied under



irradiation by visible light[8] in 10 mM phosphate buffer (pH 6.86) containing 100 mM NaCl at 0 °C (Fig. 1). Irradiation of supercoiled covalently closed circular pBR 322 DNA (form I) in the presence of **3** resulted in progressive appearance of nicked circular (form II) DNA (Fig. 1, a; lanes 2-11). Further, a close inspection of lane 5 and 6 revealed appearance of linear duplex (form III) DNA. No strand scission was observed in the absence of **3** (Fig. 1, a; lane 2) or in the dark. When the solution was thoroughly deoxygenated with argon and maintained under argon during the irradiation, the extent of strand breakage was considerably reduced compared to that observed in the aerated solution (Fig. 1, b; lane 11). On the other hand, the saturation of the reaction mixture with pure dioxygen caused little enhancement in the strand breakage. These indicate that the observed photolysis is due to photosensitized activation of dioxygen and the atmospheric concentration of dioxygen is enough to effect the DNA scission.

In order to examine whether DNA strand scission by **3** is due to the production of dioxygen-dependent free radicals, DNA cleavage was investigated with the addition of several radical scavengers (Fig. 1, b; lanes 6-10). Scavengers[5] such as D-manitol, superoxide dismutase (SOD), catalase, and NaN_3 suppress the DNA photolysis activity of **3**. These results suggest that the active oxygen species, $O_2^{\cdot-}$, ·OH, and H_2O_2, are involved in the scission. However, it has not been demonstrated that $O_2^{\cdot-}$ itself promotes DNA cleavage.

The compound **3** in aqueous solution was irradiated in the presence of excess EDTA (ethylenediaminetetraacetic acid) under argon. The light absorption by **3** in the visible region is solely due to its acridine moiety. On irradiation, the absorption spectrum clearly indicated the accumulation of one-electron reduced viologen species (Fig. 2, λ_{max}= 400 nm and 600 nm).[9] This suggests a series of events taking place, which include photoexcitation of acridine heterocycle, one electron transfer from the photoexcited acridine to the viologen moiety within the molecule, and the restoration of the oxidized acridine by sacrificial reducing agent EDTA. The photoproduction of one-electron reduced viologen from **3** supports that the superoxide species were the primary species in the DNA scission reaction since one-electron reduced viologen can give superoxide anion on reaction with dioxygen.[5] In other words, active species suspected, ·OH and H_2O_2, are presumably the secondary products generated from primary product, superoxide anion.

The photolysis of DNA did not take place in the presence of compound **2** , whereas it took place to a small extent in the presence of **1** (Fig. 1, b; lanes 3-5). In the most photosensitized DNA scissions reported, intercalating dyes are irradiated by laser beam or otherwise for a longer period of time.[1-3] It seems reasonable that the efficiency of DNA photolysis by **1** was quite low under the mild irradiation conditions of this study. The combined use of **1** and **2** indicated some scission activity, but the efficiency was the same as that of **1** (Fig. 1, b; lanes 3, 5). Obviously, a proximity of acridine and viologen functions within a molecule constitutes a critical factor in **3**.

In summary, a light-induced cleavage of DNA took place under mild conditions by using **3**. The cleavage involves superoxide species which are formed by the reaction of dioxygen with the one-electron reduced viologen structure of **3**, which in turn is introduced by a photoinitiated electron transfer from the acridine to the viologen moieties within the **3** molecule; however, the chemistry of the acridine moiety such as the lifetime and the fate of the one-electron oxidized species is yet to be studied. The proximity of the viologen and the acridine structures within **3** is the important factor for the ease of the present photocleavage reaction. Methylviologen dichloride (Paraquate) is marketed as herbicide and its activity has been discussed in terms of superoxide species that are derived from the one-electron reduced viologen species and dioxygen.[5] The present study unequivocally showed that DNA can suffer damage by such reactions. The molecular design involved in **3** seems to offer some

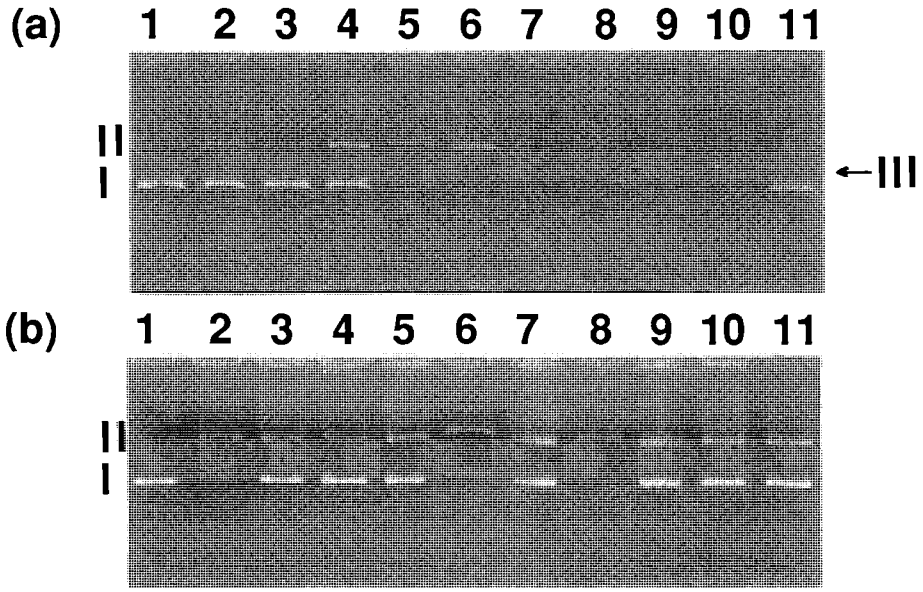


Fig. 1. Relaxation of supercoiled pBR 322 DNA by photosensitized DNA strand cleavage. (a) pBR 322 DNA (46 μM, nucleotide phosphate unit) was incubated in 10 μl of 10 mM phosphate buffer (pH 6.86) containing 100mM NaCl and various amounts of reagent **3**. The mixture was irradiated for 10 min under visible light.[8] The mixture was analyzed by 1.0% agarose gel electrophoresis (Tris-acetate EDTA buffer). The gel was stained with ethidum bromide and photographed to observe fluoresence (red filter, 320 nm excitation). Lane 1, untreated DNA (which contains both form I and form II DNAs). Lanes 2-11, treated DNAs. The concentrations of **3** were 0, 1, 5, 10, 20, 30, 40, 50, 60, and 0 μM for lanes 2-11, respectively. (b) pBR 322 DNA was tested in a similar manner to (a). Each reaction mixture contained 46 μM DNA in a total volume of 10 μl. The mixture was irradiated for 10 min under visible light. Quantination of the relaxation reaction was performed by densitometric scanning of the photographs of the ethidium-stained gels. Parentheses indicated the efficiency of the scission. Lane 1, DNA only (0); Lane 2, DNA+17 μM **3** (100); Lane 3, DNA+34 μM **1** (9.6); Lane 4, DNA+17 μM **2** (1.5); Lane 5, DNA+34 μM **1**+17 μM **2** (9.8); Lane 6, DNA+17 μM **3**+100 mM D-mannitol (8.9); Lane 7, DNA+17 μM **3**+100 ng SOD (17.5); Lane 8, DNA+17 μM **3**+100 ng catalase (54.9); Lane 9, DNA+17 μM **3**+100 ng SOD+100 ng catalase (18.2); Lane 10, DNA+17 μM **3**+100 mM NaN_3 (9.9); Lane 11, DNA+ 17 μM **3** in argon saturated aqueous solution (16.5).

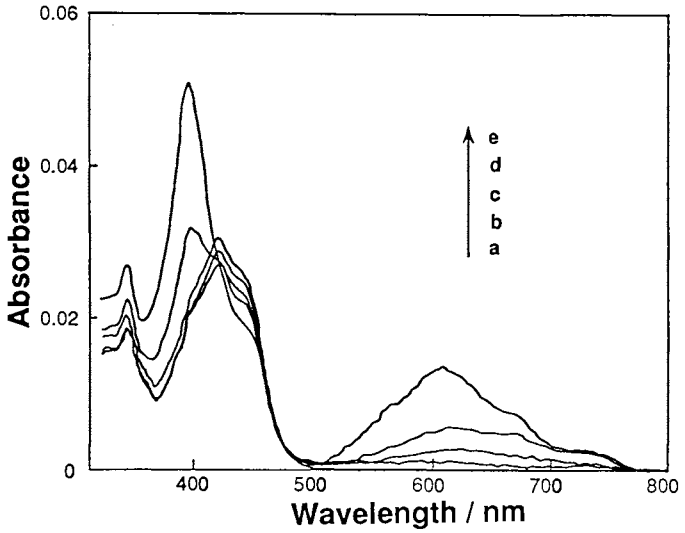


Fig. 2. Change in absorption spectra of **3** on irradiation. Compound **3** (4 μM) and EDTA (40 mM) (pH 7.5) were irradiated with visible light under argon atmosphere. Spectra were taken after (a) 0 min (no irradiation), (b) 2 min, (c) 4 min, (d) 6 min, and (e) 8 min irradiation.

new possibilities in developing DNA photocleaving reagent as well as in designing drugs of special physiological activity.

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