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# Visible Light Induced DNA Cleavage by the Hybrid Antitumor Antibiotic Dynemicin A†

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ABSTRACT: Dynemicin A, which is a hybrid antitumor antibiotic containing anthraquinone and enediyne cores, effectively breaks DNA strands upon irradiation with visible light of long wavelength. The preferential cutting sites of visible light induced DNA cleavage with dynemicin A are on the 3′-side of purine bases such as in 5′-A*T* and 5′-G*T* sequences. The observed nucleotide cutting specificity is remarkably similar to that of NADPH- (or thiol) induced DNA breakage with dynemicin A, sugesting the presence of the same DNA-cleaving intermediate. Indeed, the photoproduct of dynemicin A is chromatographically identical with the reaction product (dynemicin H) of the thiol-activated dynemicin A. On the basis of the present results, a reasonable mechanism for the visible light induced DNA cleavage of dynemicin A has been proposed.

Dynemicin A isolated from *Micromonospora chersina* is characterized as a hybrid molecule of two typical chemotypes of antitumor agents, enediyne and anthraquinone. The novel antibiotic shows a potent antitumor activity in vitro and in vivo (Konishi et al., 1989, 1990). As to the mechanism of action of dynemicin A, we recently indicated that (1) DNA strand scission by dynemicin A is significantly enhanced by addition of NADPH or thiol compounds, (2) the antibiotic interacts with the minor groove of the DNA helix, and (3) intercalation of the anthraquinone core into the DNA followed by attack of the phenylene diradical formed from the enediyne core is most likely as the mechanism of action of dynemicin A (Sugiura et al., 1990). It is believed that DNA breakage by esperamicin, calicheamicin, and neocarzinostatin is due to similar phenyl diradical species produced from their enediyne cores (Long et al., 1989; Sugiura et al., 1989; Zein et al., 1988; Myers, 1987; Goldberg, 1987). Thiol compounds such as dithiothreitol and glutathione greatly accelerate the cleavage of DNA by esperamicin, calicheamicin, and neocarzinostatin. Ultraviolet light induced DNA nicking has been observed for the cobalt, manganese, and iron complex systems of bleomycin (Suzuki et al., 1985). In the case of esperamicin or neocarzinostatin, irradiation with ultraviolet light is also effective in causing nicks in supercoiled DNA (Uesawa et al., 1989). In this paper we report that visible light induces remarkable DNA cleavage by dynemicin A and that the photoproduct of dynemicin A is chromatographically identical with the reaction product (dynemicin H) of dynemicin A and thiol compounds.

## Materials and Methods

*Drugs and Chemicals.* Purified dynemicins A, L, and H (Figure 1) were kindly supplied by Bristol-Myers Research Institute, Tokyo, Japan. Plasmid pBR322 DNA was isolated from *Escherichia coli* C600, and restriction endonucleases *Eco*RI and *Hae*III were obtained from Takara Shuzo (Kyoto, Japan). G4 DNA obtained from phage R199/G4 ori replicative form DNA was a kind gift of T. Komano (Kyoto University, Kyoto, Japan). NADPH and 4-hydroxythiophenol were purchased from Sigma. All other chemicals used were of commercial reagent grade.

*Assay for DNA Cleavage Activity.* Analysis of drug-induced damage to supercoiled, covalently closed, circular (form I) pBR322 DNA was performed under irradiation of visible light. The reaction samples (total volume 20 μL) contained 20 μM dynemicin A, 0.4 μg of pBR322 plasmid DNA, and 20 mM Tris-HCl buffer (pH 7.5) and were irradiated at a distance of 10 cm by a commercial 300-W photoreflector lamp (Toshiba, Tokyo; color temperature of 3150 K) for 30 min at 4 °C. To investigate the dependence on wavelength of visible light for DNA cleavage activity, various photofilters were used. UV, Y2, R1, and R64 filters (Kenko, Tokyo) approximately cut off wavelengths shorter than 360, 460, 580, and 610 nm, respectively. The reactions were stopped by addition of cold ethanol (60 μL) and 0.3 M sodium acetate, then the samples were immediately chilled at −70 °C in a dry ice/ethanol bath, and the DNA was recovered by ethanol precipitation. Each of the samples was dissolved in 20 μL of loading buffer containing 0.05% bromophenol blue and 10% glycerol and heated for 1 min at 60 °C before electrophoresis. Electrophoresis was peformed on a 1% agarose gel containing ethidium bromide (0.5 μg/mL).

*Nucleotide Sequence Analysis.* The reaction samples (total volume 20 μL) contained a 5′ or 3′-end-labeled 100-base-pair (bp) (*Eco*RI–*Hae*III) G4 gene F/G space fragment, sonicated calf thymus carrier DNA (5 μg/mL), and 20 mM Tris-HCl buffer (pH 7.5). Nucleotide sequence cleavage was performed by addition of dynemicin A (20 μM) and NADPH (1 mM) or 4-hydroxythiophenol (1 mM) for 6 h at 37 °C or by irradiation of visible light through the UV filter for 30 min at 4 °C. The condition of light exposure was the same as described

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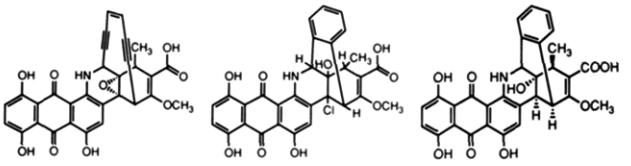

FIGURE 1: Chemical structures of dynemicin A (left), dynemicin L (middle), and dynemicin H (right).

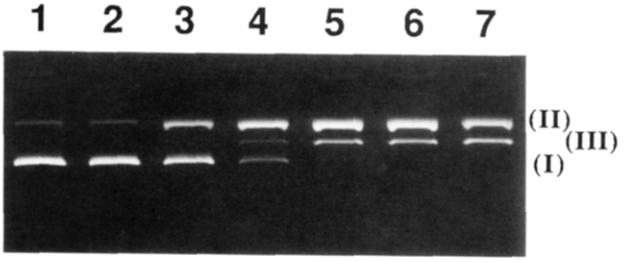


FIGURE 2: Agarose gel electrophoretic patterns of DNA nicking by dynemicin A (20 $\mu$M) with irradiation from a 300-W photoreflector lamp for 30 min at 4 °C. The reaction samples were irradiated through various photofilters, namely, 610-nm- (lane 4), 580-nm- (lane 5), 460-nm- (lane 6), and 360-nm- (lane 7) cutoff filters. Lane 3 indicates DNA cleavage by dynemicin A (20 $\mu$M) in the absence of visible light radiation (lane 3). Lane 1 shows intact DNA alone, and lane 2 presents DNA irradiated through a 360-nm-cutoff filter in the absence of dynemicin A.

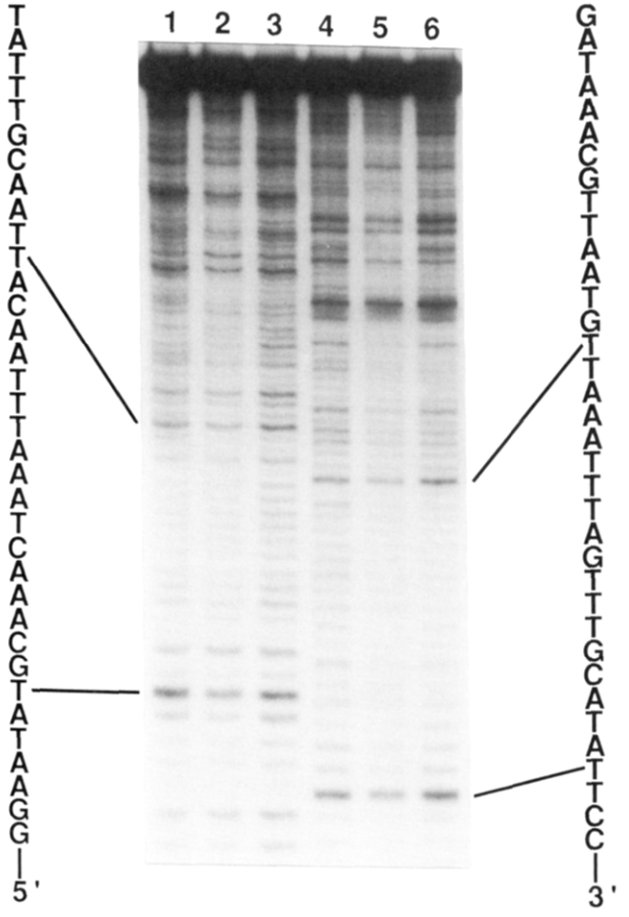


FIGURE 3: Strand scission of 5′-end-labeled (lanes 1–3) and 3′-end-labeled (lanes 4–6) DNAs by dynemicin A (20 $\mu$M) in the presence of visible light radiation (lanes 1 and 6), 4-hydroxythiophenol (lanes 2 and 5), and NADPH (lanes 3 and 4). The experimental conditions are described under Materials and Methods.

under Assay for DNA Cleavage Activity. Cold ethanol was then added to the samples to stop the reaction. Electrophoresis was performed on a 10% polyacrylamide/7 M urea slab gel, and DNA sequencing was carried out by the Maxam–Gilbert method (Maxam & Gilbert, 1980). The autoradiograms were scanned with a laser densitometer (LKB Model 222 UltroScan XL).

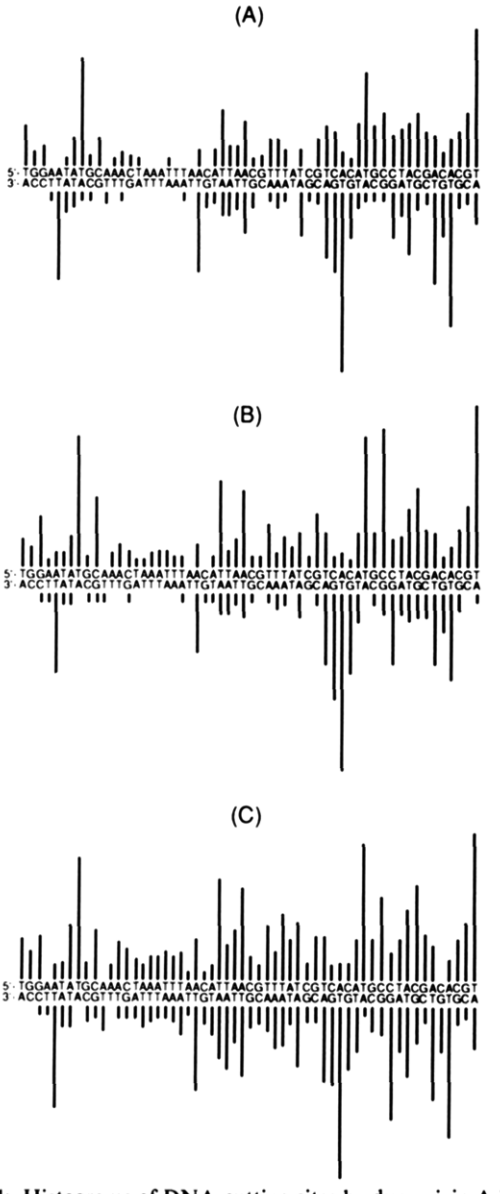


FIGURE 4: Histograms of DNA-cutting sites by dynemicin A in the presence of visible light radiation (A), 4-hydroxythiophenol (B), and NADPH (C). Relative DNA cleavage frequencies were obtained from densitometric scans of gel autoradiograms.

*HPLC of Light-Irradiated Dynemicin A.* After irradiation for 15 or 30 min at room temperature, the reaction product of dynemicin A (20 $\mu$M) was separated by high-performance liquid chromatography (HPLC) on an A301-3S-3-120A ODS column (Yamamura Kagaku, Kyoto, Japan; 4.6 × 10 mm) with methanol–0.15% $KH_2PO_4$ (pH 3.5) (75:25 v/v) as the solvent. The eluate was monitored at 569 nm.

## Results

*Visible Light Induced DNA Nicking by Dynemicin A.* Figure 2 shows typical gel electrophoretic patterns for visible light induced strand scission of superhelical DNA by dynemicin A. In the presence of 360-nm-cutoff light radiation (lane 7), the antitumor antibiotic evidently converted covalently closed, supercoiled (form I) pBR322 DNA to nicked circular (form II) and linear duplex (form III) DNAs. The DNA-cleaving activity of dynemicin A induced by 460- or 580-nm-cutoff light (lanes 6 and 5) was almost the same as that by

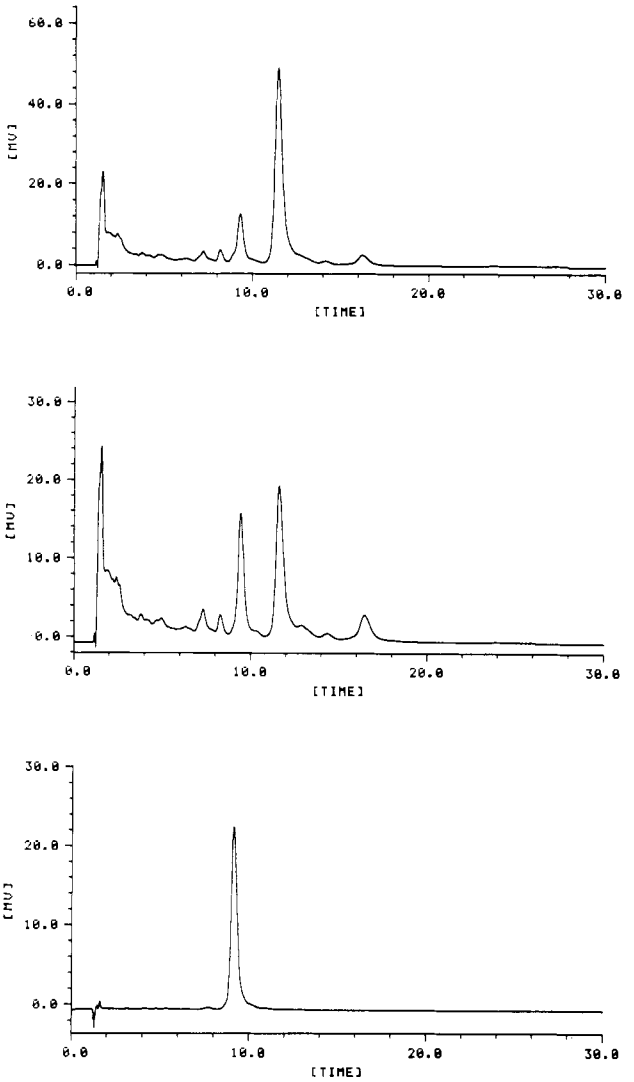


FIGURE 5: HPLC analyses of light-irradiated dynemicin A for 15 min (upper) and 30 min (middle) and of standard dynemicin H (lower).

360-nm-cutoff light. On the other hand, irradiation by 610-nm-cutoff light (lane 4) was unable to activate dynemicin A as much as the above-mentioned wavelength-cutoff light. Of course, it should be noted that dynemicin A caused only weak DNA strand scission in the absence of light (lane 3).

*Sequence-Specific DNA Cleavage by Visible Light Activated Dynemicin A.* Cleavage data for 5′- and 3′-end-labeled DNA strands in the dynemicin A–visible light system are presented in Figure 3. The same DNA fragments were treated with dynemicin A–NADPH or dynemicin A–4-hydroxythiophenol systems, and the DNA cutting sites were compared with the visible light induced DNA cleavage pattern. In this experiment, the *Eco*RI–*Hae*III G4 gene F/G space fragment (100 bp) which contains A,T-rich sequences was used as the DNA substrate. As shown in the histograms of Figure 4, the visible light induced DNA cutting mode of dynemicin A is remarkably similar to the reductant-induced DNA cleavage pattern of the antibiotic. In the three activation systems, dynemicin A preferentially attacks the 3′-side of purine bases such as in 5′-A*T* and 5′-G*T*. In addition, the drug appears to cause typical double-strand cuts at the following sequences:

5′-ATA*T* 5′-ACA*T* 5′-ACG*T*

3′-*T*ATA 3′-*T*GTA 3′-*T*GCA

*Identification of Photoproduct of Dynemicin A by HPLC.* As clearly shown in Figure 5, HPLC analysis of the light-irradiated mixture revealed the formation of one major photoproduct (retention time 9.3 min), and analysis confirmed a substantial decrease of the starting dynemicin A (retention time 11.4 min). The reaction product of dynemicin A with methyl thioglycolate, which was isolated by HPLC purification and assigned as dynemicin H on the basis of spectral data (Konishi et al., unpublished data), gave a retention time identical with that of this photoproduct in the present HPLC analysis. Therefore, the photoproduct of dynemicin A can be chromatographically identified with dynemicin H.

## Discussion

We found that dynemicin A shows potent DNA breakage activity in the presence of visible light radiation and that this system is more effective than the NADPH or thiol activation of dynemicin A. Figure 2 reveals that the antibiotic can be effectively activated even by 580-nm-cutoff light, namely, red light. The DNA strand scission of dynemicin A induced by 580-nm-cutoff light is equivalent to that by 360-nm-cutoff light. Light-induced DNA strand breaks by antibiotics such as certain metallobleomycins and esperamicin, are effective only with UV light or near-UV light (Chang & Meares, 1982, 1984; Sugiura et al., 1982; Suzuki et al., 1985; Uesawa et al., 1989). Of special interest is the fact that dynemicin A has strong DNA cleavage activity in the presence of low-energy visible light. Indeed, dynemicin A presents broad absorptions

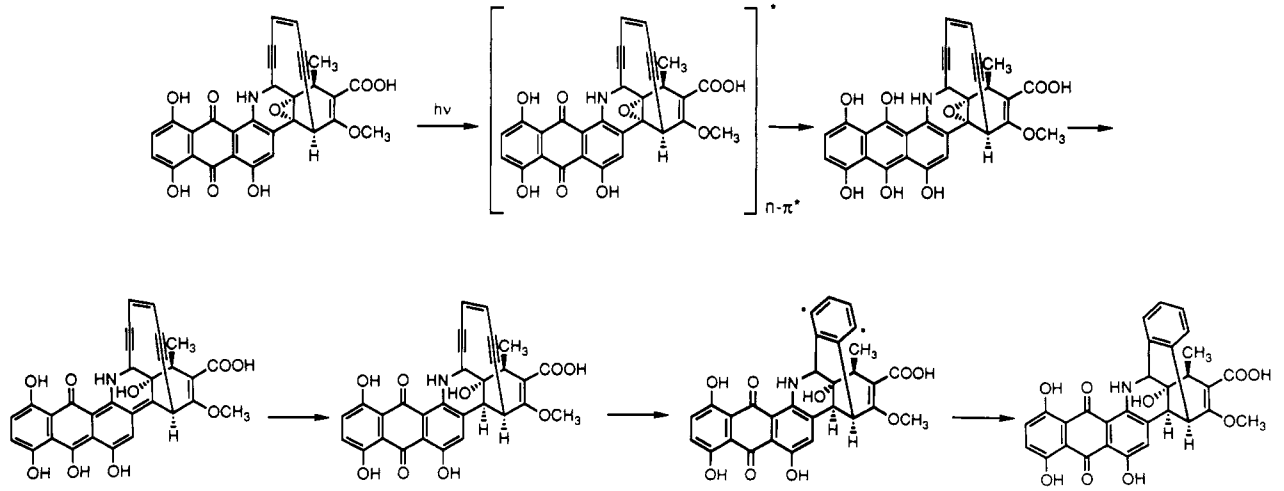


FIGURE 6: A probable mechanism for photoactivation of dynemicin A.

between 500 and 630 nm which are probably due to the anthraquinone core (Konishi et al., 1989, 1990). In 10% methanol–90% 20 mM Tris-HCl buffer (pH 7.5), maximal absorptions of dynemicin A were observed at 560 nm ($\epsilon$ 5100) and 530 nm ($\epsilon$ 5200) in the absence of DNA and at 565 nm ($\epsilon$ 4800) and 532 nm ($\epsilon$ 4960) in the presence of DNA. Apparently, the present light-induced DNA cleavage reaction correlates with the absorption spectrum of the antibiotic. Dynemicin A was irreversibly inactivated by visible light exposure for 1 h. We demonstrated that dynemicin A is converted to dynemicin H by the light irradiation. Dynemicin derivatives such as dynemicins L and H in which the enediyne core was aromatized showed no DNA cleavage activities by the irradiation with visible light or UV light. The results indicate that the light-induced DNA breakage is not directly due to the anthraquinone core of the antibiotic but to the enediyne core.

Figure 6 displays a probable mechanism for the light-induced DNA breakage by dynemicin A. As proposed in NADPH- or thiol-activated dynemicin A, conversion of the quinone into the hydroquinone is the first key step for drug activation (Sugiura et al., 1990; Semmelhack et al., 1990). It is well-known that quinones such as *p*-benzoquinone and 1,4-naphthoquinone are capable of undergoing photoreduction through $n-\pi^*$ transition states (Maruyama et al., 1973, 1974). Therefore, the anthraquinone of dynemicin A appears to be converted into the hydroquinone by photoreduction. As in the NADPH- or thiol-induced DNA cleavage reaction of dynemicin A, the result is the conversion of the enediyne core to 1,4-dehydrobenzene diradical through the Bergman rearrangement. The radical species is responsible for the DNA-cleaving property of dynemicin A and suggests the presence of the same DNA-cleaving intermediate in the light-, NADPH-, and thiol-activated dynemicin A reactions. Indeed, the dynemicin-mediated DNA-cutting modes are significantly similar in these three systems. Further, in the experiment of short light irradiation for dynemicin A, we detected an organic free radical by ESR spin trapping using 5,5′-dimethyl-1-pyrroline *N*-oxide (DMPO) (data not shown). A similar free radical has also been observed in the esperamicin–UV light system (Uesawa et al., 1989). Finally, the photoproduct of dynemicin A is the aromatized compound dynemicin H.

The preferred cutting sites in the light-induced DNA degradation of dynemicin A are on the 3′-side of purine bases such as 5′-A*T* and 5′-G*T*. This nucleotide cleaving specificity is remarkably similar to that of the dynemicin A mediated DNA breakage in the presence of NADPH or 4-hydroxythiophenol. Dynemicin A also causes strong strand breaks 3 bp apart in the two DNA strands, which are characteristic of double-stranded DNA breakage. The asymmetric cleavage pattern on the 3′-side of opposite strands suggests interaction of the antibiotic with the minor groove of the DNA helix (Sluka et al., 1987). These results are consistent with the previous study by NADPH- and thiol-activated dynemicin A (Sugiura et al., 1990). The DNA-cleaving specificity of dynemicin A for the present A,T-rich fragment is relatively higher than that for other DNA fragments. Dynemicin A may be a useful reagent for probing some higher ordered structures of DNA, because A,T-rich sequences are known to have unusual DNA structure such as curved DNA (Trifonov & Ulanovsky, 1988).

In conclusion, light in the visible region is very effective at stimulating DNA nicking by dynemicin A. The action spectrum correlates with the absorption spectrum of dynemicin A in the wavelength range between 350 and 600 nm. Probably, photoreduction of the anthraquinone core contributes to activation of the enediyne core of dynemicin A. The light-activated dynemicin A preferentially attacks the 3′-side of purine bases. This cutting mode is almost the same as that of NADPH- or thiol-activated dynemicin A, suggesting a similar activation mechanism of dynemicin A in the presence of visible light, NADPH, and 4-hydroxythiophenol.

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