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Photo-Induced DNA Cleavage Reaction Characteristics of Propargylic Sulfones Possessing Anthraquinone Chromophore

Ken-ichi Haruna,[a] Kazuhito Tanabe,[a] Atsushi Ishii,[a] Wei Min-Dai,[b] Hiroshi Hatta[a] and Sei-ichi Nishimoto[a,*]

[a]*Department of Energy and Hydrocarbon Chemistry, Faculty of Engineering, Kyoto University, Kyoto University Katsura, Nishikyo-ku, Kyoto 615-8510, Japan*

[b]*Department of Chemistry, The Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong, SAR, China*

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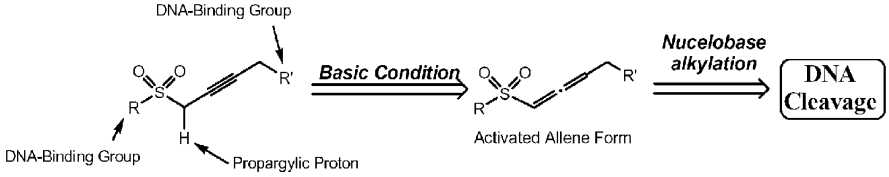
Abstract—DNA cleavage potency of propargylic sulfones possessing anthraquinone chromophore **1** under UV-irradiation was evaluated in comparison with the dark reaction. **1** showed inefficient DNA cleavage activity, while having considerably strong DNA binding ability. This result is accounted for by spatial conditions that the activated alkylating allenic site of intercalated **1** could not effectively approach to DNA bases, most probably guanine moiety, and thereby led to insufficient DNA strand cleavage. In contrast, the DNA cleavage activity of **1** was notably enhanced upon UV-irradiation ($\lambda_{ex} = 365$ nm) followed by incubation. Under UV-irradiation, further DNA cleavage were occurred primary at 5′-G of GG steps within DNA. A DNA cleavage mechanism for **1**, by which photo-induced one-electron oxidation of 5′-G of GG steps may occur along with ordinary alkylation, has been proposed.

Introduction

Alkylation of nucleobase by nucleophile is one of the mechanisms for DNA strand cleavage and a variety of antibiotics have been reported to cleave DNA by such an alkylation mechanism, involving interactions with DNA through various binding modes as a primary step.[1–4] Propargylic sulfones are pH-dependent nucleobase alkylating agents as identified firstly by Nicolau's group.[5] A proposed mechanism of DNA cleavage involves intercalation of DNA-binding groups and isomerization of propargylic sulfones into allenic sulfones under basic conditions, followed by nucleophilic addition of nucleobase, especially guanine base, toward allenic sulfones (Scheme 1).

To establish guides for molecular design of propargylic sulfones with higher DNA cleavage ability, we have characterized DNA cleavage reactivity of propargylic sulfones possessing anthraquinone chromophore that is widely used as a DNA intercalator[7–11] and a photooxident leading to DNA cleavage by one-electron oxidation (Chart 1).[12–19] Introduction of such a planar molecular group provided propargylic sulfones with sufficient intercalating ability, but not with effective DNA cleavage activity.[11] This result suggests that spatial arrangement of activated allene against nucleophilic DNA bases, most probably guanine moiety, is unfavorable for alkylation causing DNA cleavage. In contrast, propargylic sulfone possessing anthraquinone chromophore showed high DNA



Scheme 1. Proposed mechanism of DNA cleavage by propargylic sulfones.

*Corresponding author. Tel.: +81-75-383-2500; fax: +81-75-383-2501; e-mail: nishimot@scl.kyoto-u.ac.jp

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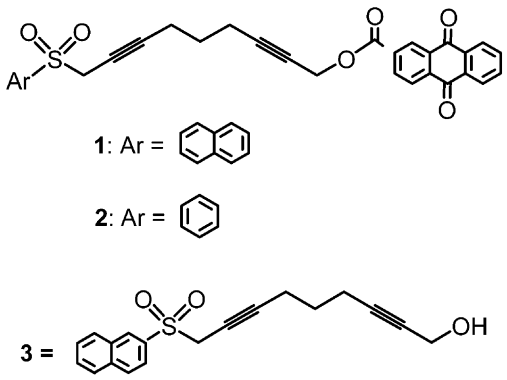

Chart 1. Molecular structures of propargylic sulfone.

cleavage activity under conditions of UV-irradiation followed by incubation. The analysis of DNA cleavage site revealed that DNA was cleaved primarily at 5′-G of GG steps upon UV-irradiation. This enhanced DNA cleavage seems to be induced mainly by one-electron photo-oxidation in addition to ordinary alkylation.[20–22]

Results and Discussion

Initially, we determined the inhibitory binding constants (K') of propargylic sulfones **1**–**3** against intercalated ethidium bromide (EB) using Salmon Sperm DNA according the reported fluorometry (Table 1).[23] An aliquot of EB solution was used to titrate the Salmon Sperm DNA in 5% DMSO containing H_3BO_3/NaOH buffer solution (pH 8.5) at various concentrations of a given propargylic sulfone with recording the change of fluorescence intensity at maximum emission wavelength (586 nm in this study) upon excitation at 525 nm.

Table 1. Binding constants (K') of propargylic sulfones

Compd	$K' \times 10^{-4}/M^{-1}$
1	3.7
2	1.7
3	3.2
Naphthalene	5.2
Anthraquinone	11.3

As shown in Table 1, propargylic sulfone **1** showed about 2-fold higher intercalating ability than an analogue **2**, indicating that the naphthyl group is a much better DNA intercalator than the phenyl group.[11] In addition, propargylic sulfone **1** showed about 1.2-fold higher intercalating ability relative to another analogue **3**. This result indicates that not only naphthalene chromophore but also anthraquinone chromophore of **1** can intercalate into duplex DNA. Thus, it is clear that introduction of naphthalene and/or anthraquinone to propargylic sulfones is effective for enhancing their intercalating ability.

DNA cleavage by the propargylic sulfone **1** by reference to its analogue **3** (without anthraquinone chromophore) was also examined using agarose gel electrophoresis of restriction fragment of circular ΦX174 RFI DNA.[11]

The gel picture recorded after UV-irradiation and incubation of propargylic sulfone **1** and **3** with DNA is shown in Figure 1(a), from which relative DNA cleavage activities of **1** and **3** were evaluated [Fig. 1(b)]. It is seen in Figure 1(a), that **1** resulted in less efficient DNA cleavage than that of **3** in the dark (compare lanes 4 and 7). This result is opposed to DNA binding constants shown in Table 1. Binding constants of naphthalene ($K' = 5.2 \times 10^4\,M^{-1}$) and anthraquinone ($K' = 11.3 \times 10^4\,M^{-1}$) suggest that **1** favors intercalation of anthraquinone chromophore into DNA rather than naphthalene chromophore. As shown in Figure 2, activated allenic site is located far from nucleobases upon intercalation of one anthraquinone chromophore of **1** into DNA [Fig. 2(a)], while being otherwise closer to nucleobase for alkylation upon

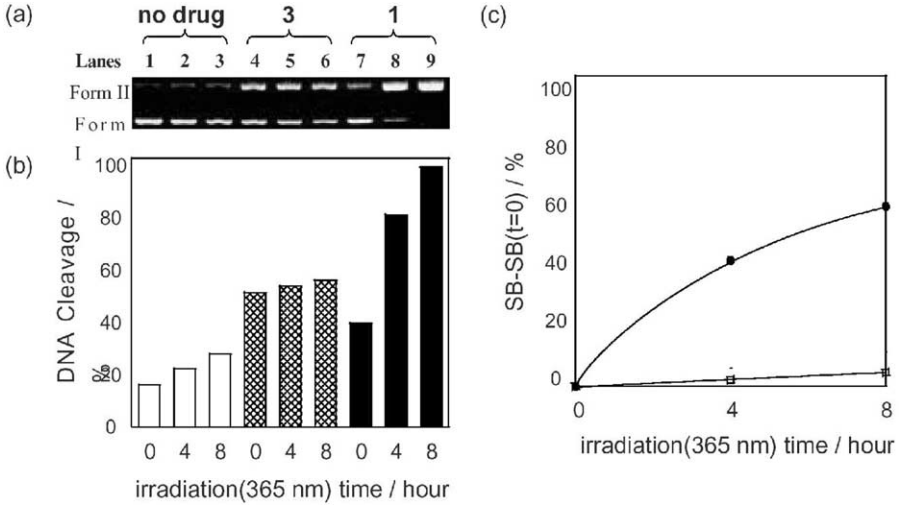

Figure 1. DNA cleavage by propargylic sulfones **1** and **3**: (a) 1% agarose gel electrophoresis. ϕX174 RFI DNA (50.0 μM) was incubated with propargylic sulfone **1** and **3** at 10 μM as observed after UV-irradiation followed by incubation in 20% DMSO-containing TAE buffer solution (pH 8.5) at 37 °C for 72 h and then analyzed by gel electrophoresis and ethidium bromide stain. (b) the scanning densitometry results of gel pictures; (c) efficiencies of DNA cleavage by propargylic sulfone **1** and **3** upon UV-irradiation followed by incubation, in which SB represents strand breaks occurred at a given UV-irradiation time: (●) **1**, (□) **3**.

intercalation of the naphthalene chromophore (Fig. 2b). Since intercalations of anthraquinone and naphthalene moieties of **1** should occur competitively, **1** would show less efficient DNA cleavage activity than **3** which intercalates into DNA almost preferentially from naphthalene chromophore. It is thus noted that appropriate spatial arrangement between activated allenic site and nucleobase should be the most important factor determining the DNA cleavage by a nucleobase alkylation mechanism.[11]

DNA cleavage by propargylic sulfone **1** was further investigated upon UV-irradiation and incubation. It is well known that anthraquinone derivatives are light-activated agents that oxidize DNA bases.[12–19] Accordingly, under UV-irradiation conditions, **1** is expected to cause DNA cleavage by not only the nucleobase alkylation mechanism but also the oxidation mechanism derived from electronic excitation of anthraquinone chromophore. In accord with this expectation, **1** became apparently more efficient in DNA cleavage in combination with UV-irradiation, relative to the activity in the dark [Fig. 1(c)]. This result indicates that combination of alkylation with photo-induced oxidation results in more efficient DNA cleavage. The quantum yield for the photo-induced DNA cleavage by **1** was measured to be 2.2×10^{-5} by means of chemical actinometer using potassium ferrioxalate.[24–26]

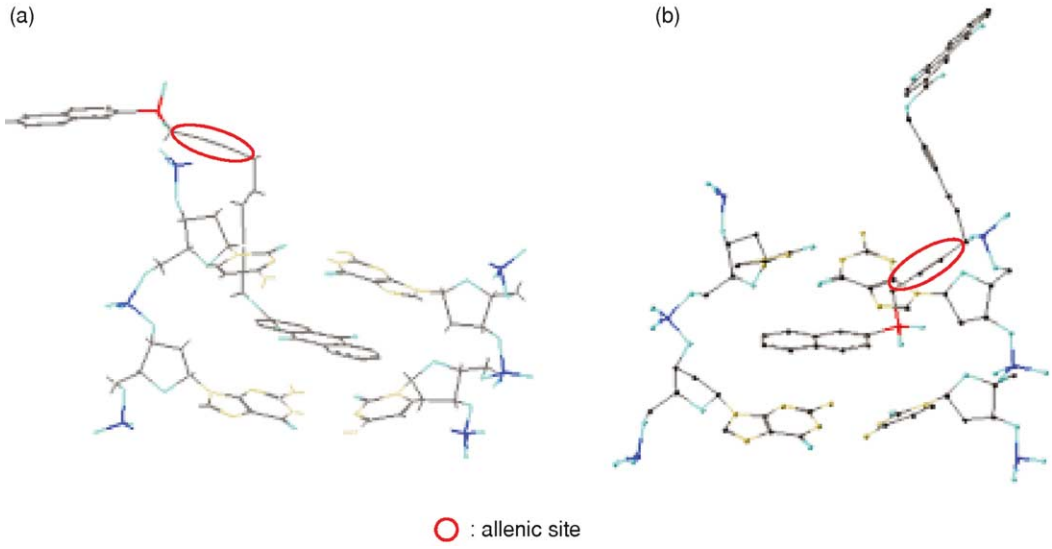

Figure 2. Binding models of propargylic sulfone possessing naphthalene and anthraquinone chromophores **1** bound to duplex DNA: (a) intercalation of naphthalene chromophore; (b) intercalation of anthraquinone chromophore.[27]

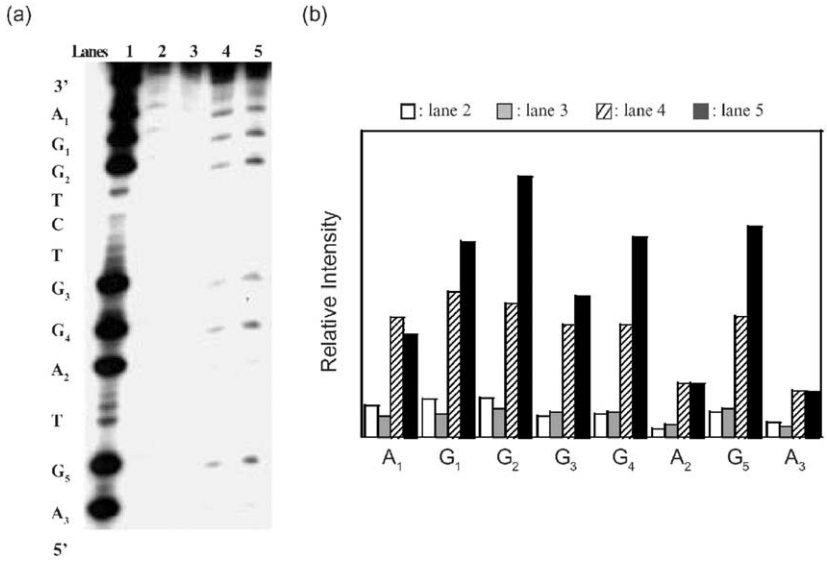

Figure 3. (a) An autoradiogram of a denaturing sequencing gel for the ^{32}P-5′-end labeled 21-mer DNA after alkylation and photooxidation in the presence of propargylic sulfone **1**. Duplex DNA was cleaved by **1** (100 μM) in 20% DMSO-containing TAE buffer solution (pH 8.5). After piperidine treatment (90 °C, 20 min), the samples were electrophoresed through a denaturing 15% polyacrylamide/7 M urea: lane 1, Maxam-Gilbert G + A sequencing reactions; lane 2, control DNA, with piperidine treatment only; lane 3, neither UV-irradiated nor incubated in the presence of **1**; lane 4, incubated for 72 h at 37 °C; lane 5, UV-irradiated at $\lambda_{ex}=365$ nm for 30 min, followed by incubation similarly. (b) The histogram representing relative intensities of cleavage bands obtained by densitometry assay of lanes 2, 3, 4 and 5 in (a).

The base selectivity in the dark and photo-induced DNA cleavage reactions by propargylic sulfone **1** was investigated using polyacrylamide gel electrophoresis of ^{32}P-5′-end labeled 21-mer DNA. The UV-irradiated and incubated sample solutions containing DNA and **1** were treated with hot piperidine to visualize alkaline labile damage. The autoradiograms recorded for the samples thus treated are shown in Figure 3a.

A close inspection of lane 3 in Figure 3(a) revealed that propargylic sulfone **1** could not cause any DNA cleavage with neither UV-irradiation nor incubation. As shown in Figure 3(a), **1** caused DNA cleavage at every guanine bases upon the incubation alone at 37 °C in the dark [lane 4 in Fig. 3(a)]. This result supports a mechanism that **1** cleaves DNA by the alkylation mechanism at guanine bases.[6]

Moreover, enhancement of DNA cleavage at 5′-G of GG sequences by propargylic sulfone **1** was observed under UV-irradiation conditions followed by incubation [lane 5 in Fig. 3(a)]. It is well known that 5′-G of 5′-GG-3′ is a sink in hole migration through DNA; i.e., an electron-loss center created in B-form DNA would end up predominantly on 5′-G of GG sequences.[12–19] In this view, it is most likely that **1** cleaves DNA by one-electron oxidation and alkylation mechanism under UV-irradiation condition followed by incubation. A control experiment was also carried out under conditions of UV-irradiation alone without follow-up incubation, indicating occurrence of DNA cleavage characteristic of a one-electron oxidation mechanism (data not shown). It is therefore suggested that the alkylation and the photo-induced one-electron oxidation proceed independently to result in DNA cleavage under the present conditions.

Conclusion

In this work, we evaluated DNA cleavage characteristics of propargylic sulfones possessing anthraquinone chromophore. This compound showed higher intercalating ability, but not expected efficient DNA cleavage activity. This is attributable to unfavorable spatial arrangement of activated allenic site against guanine base for alkylation reaction in the intercalation of anthraquinone chromophore. Therefore, it is clear that spatial arrangement of activated allenic site is extremely important for DNA cleavage by an alkylation mechanism. Because of the photo-induced oxidizing ability of anthraquinone chromophore, apparent DNA cleavage activity of **1** was remarkably enhanced upon UV-irradiation. The enhanced DNA cleavage occurred primarily at 5′-G of GG sequences, indicating that guanine bases undergo more readily photo-induced one-electron oxidation by anthraquinone chromophore. In conclusion, propargylic sulfone possessing anthraquinone chromophore can cleave DNA efficiently by a photo-induced one-electron oxidation mechanism, in supplement to an ordinary alkylation mechanism.

Experimental

Materials

Syntheses of propargylic sulfones were performed as reported previously.[11] Anthraquinone and naphthalene were of the best available grades purchased from Nacalai Tesque and used without further purification. Salmon Sperm DNA and supercoiled ΦX174 RFI DNA were used as received from Gibco BRL and Takara Shuzo Co., Ltd, respectively. The oligo-deoxynucleotides, T4 polynucleotide kinase, and γ-[^{32}P]-ATP (10 mCi/mL) were used as received from Invitrogen, Nippon Gene (10 unites/μL), and Amersham, respectively.

Instrumentation

Fluorescence spectra were recorded on a Hitachi F-2000 and Shimadzu RF-5300PC. Photoirradiation at 365 nm was carried out using a Ultra-Violet Products NTFL-40 transilluminator. An ATTO sequencing gel electrophoresis apparatus and a Gibco BRL Model S2 sequencing gel electrophoresis apparatus were used for agarose gel electrophoresis and polyacrylamide gel electrophoresis (PAGE), respectively. The gels were analyzed by densitometry with an Atto Lane Analyzer (version 3).

Measurements of DNA binding ability

An aliquot of approximately 300 μM ethidium bromide (EB) solution was used to titrate the Salmon Sperm DNA in 5% DMSO-containing H_3BO_3/NaOH buffer solution (pH 8.5) at various concentrations of an propargylic sulfone with recording the change of fluorescence intensity at maximum emission wavelength with the excitation at 525 nm.

Intrinsic and observed DNA binding constants, K and K_{obs}, at various concentrations of propargylic sulfones bearing either anthraquinone or naphthalene chromophore were evaluated from the slope of the Scatchard plot for the binding of EB to DNA in the absence and presence of propargylic sulfones. The relationship between K and K_{obs} is shown in eq 1:

$$\frac{1}{K_{obs}} = \frac{1}{K} + \left(\frac{K}{K'}\right) X C \quad (1)$$

where K' is the inhibitory DNA binding constant of the propargylic sulfones, anthraquinone and naphthalene and C is the concentration of those unbound compounds that are approximately equal to the total concentration before titration of EB. The value of K' was calculated from a plot of $1/K_{obs}$ versus C.

Measurements of DNA cleavage activity

DNA cleavage studies on the propargylic sulfones were performed by the use of supercoiled, covalently closed, circular ΦX174 RFI double-stranded DNA (Form I). Solution of 50.0 μM/bp (micromolar per base pair) solution of ΦX174 RFI DNA and 10 μM various pro-

pargylic sulfones in 20% DMSO-containing TAE buffer (pH 8.5) (total volume 10 μL) was UV-irradiated with a transilluminator ($\lambda_{ex}=365$ nm) at 37 °C. Reaction mixtures after UV-irradiation were incubated at 37 °C for 72 h and then analyzed by gel electrophoresis (1% agarose gel, ethidium bromide stain). DNA cleavage was indicated by the formation of relaxed circular DNA (Form II) and linearized DNA (Form III). The gels were placed on a UV transilluminator ($\lambda_{ex}=365$ nm) and photographed with Polaroid 667 film. The relative densities of various DNA bands on a picture were quantified by densitometry with an ATTO Lane Analyzer. The percentage of DNA cleavage was calculated by following eq. 2:

$$\text{DNA cleavage}/\% = \frac{[\text{Form II}] + 2[\text{Form III}]}{[\text{Form I}] + [\text{Form II}] + 2[\text{Form III}]} \quad (2)$$

In order to evaluate the percentage of DNA cleavage with good accuracy, the assays in the present study were performed under conditions that concentrations of propargylic sulfones were adjusted so as not to induce Form III.

Analysis of DNA cleavage sites

The oligo-deoxynucleotide (ODNs, 400 pmol strand concentration) was 5′-end-labeled by phosphorylation with 4 μL of [γ-^{32}P]ATP and 4 μL of T4 poly nucleotide kinase using stranded procedures.[28,29] The 5′-end-labeled ODNs were recovered by ethanol precipitation and further purified by 15% preparative denaturing gel electrophoresis and isolated by the crush and soak method.[30] ^{32}P-5′-end-labeled ODNs were hybridized to the complementary stranded (2.0 μM, strand concentration) in TAE buffer at pH 8.5. Hybridization was achieved by heating the sample at 90 °C for 5 min and slowly cooling to room temperature. The ^{32}P-5′-end-labeled ODN duplex containing propargylic sulfones (total volume 15 μL) was irradiated with a transilluminator at 37 °C, and then incubated at 37 °C. Reaction mixtures after incubation were precipitated by 800 μL ethanol precipitated with the addition of 10 μL of 3 M sodium acetate, 10 μL of 1 mg/1 mL Herring sperm DNA. The DNA thus precipitated was washed with 100 μL of 80% cold ethanol and dried in vacuo. The purified DNA was resolved in 50 μL of water or 50 μL of 10% piperidine (v/v), heated at 90 °C for 20 min, and evaporated under reduced pressure. The radioactivity of the samples was then assayed using an Aloka 1000 liquid scintillation counter and the dried DNA pellets were resuspended in 80% formamide loading buffer (a solution of 80% v/v formamide, 1 mM EDTA, 0.1% xylene cyanol, and 0.1% bromophenol blue). All reactions, along with Maxam-Gillbert G+A sequencing reactions,[28,29] were heat-denatured at 90 °C for 3 min and quickly chilled on ice. The samples (0.8–1.0 μL, 5–20×103 cpm) were loaded onto 12% polyacrylamide/7 M urea sequencing gels and electrophoresed at 1900 V for about 2 h and transferred to a cassette and stored at −80 °C with Fuji X-ray film (RX-U). The gels were analyzed by densitometry with an ATTO Lane Analyzer (version 3). The intensities of the spots resulting from piperidine treatment were determined by volume integration.

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