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SINGLE-STRAND CLEAVAGE OF DNA WITH SITE-SPECIFICITY BY PHOTOLYSIS OF AZULENEQUINONES

Tetsuo Nozoe,† Chun Chieh Lin,[a,b] Shwu-Chen Tsay,[a] Sheng-Fa Yu,[a,b] Lung Ching Lin,[a] Paw Wang Yang, and Jih Ru Hwu*,[a,b]

[a]*Organosilicon and Synthesis Laboratory, Institute of Chemistry, Academia Sinica, Nankang, Taipei, Taiwan 11529, Republic of China; and*

[b]*Department of Chemistry, National Tsing Hua University, Hsinchu, Taiwan 30043, Republic of China*

Abstract: Single-strand cleavage of DNA in a phosphate buffer at pH 6.0 was accomplished by photolysis of various 1,5- and 1,7-azulenequinones with 350-nm UV light; results from the analysis of a ^{32}P-end-labeled DNA fragment obtained by gel electrophoresis indicate the predominant cleavage occurring at the guanine residue. © 1997 Elsevier Science Ltd.

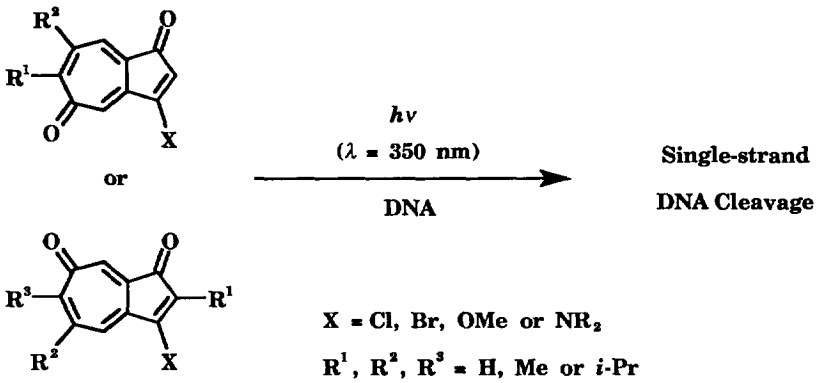
Quinones, such as 2-methyl-1,4-naphthoquinone, can function as sensitizers in the single-strand breaks of supercoiled DNA under photolytic conditions.[1,2] Irradiation of naphthoquinones gives transient and highly electrophilic quinone methides, which conjugated with an oligonucleotide can alkylate DNA with sequence-specificity.[3] On the other hand, upon enzymic one- or two-electron reduction, redox-active quinones including doxorubicin and anthracyclines react with DNA, RNA, or proteins to give alkylated biomolecules.[4,5] Quinones of azulene (i.e., azulenequinones) are non-benzenoids; some of those cytotoxic compounds have been tested against P-388 leukemia in mice.[6] To the best of our knowledge, their capability in cleaving DNA has never been documented. Herein we report our new findings on the use of UV light as the trigger for cleavage of DNA by 1,5- and 1,7-azulenequinones (Scheme 1). Site-specificity was obtained at the guanine residue by analysis of the autoradiogram from denaturing polyacrylamide gel electrophoresis.

Most quinones known today are benzoquinones or polybenzenoid hydrocarbons.[6] The two carbonyl groups in biologically active quinones are often separated by one –C=C– unit from each side of a cyclohexadiene unit. Among the possible 16 azulenequinone isomers,[6] 1,5- and 1,7-azulenequinones are predicted by Scott, Houk, et al.[7] to possess the greatest chemical stability. The two carbonyl groups therein are separated by two –C=C–C=C– units on each side in the azulene nucleus of 1,5-azulenequinones, yet by one

†Deceased April 4, 1996; Tokyo Research Laboratories, Kao Corporation, 2-1-3, Bunka, Sumida-ku, Tokyo 131, Japan

–C=C–C=C–C=C– unit and one –C=C– unit in 1,7-azulenequinones. Results from our molecular mechanics calculations indicate that the direct distance between the two carbonyl groups were 6.84 Å for 1,5-azulenequinones and 5.24 Å for 1,7-azulenequinones, which are longer than or close to 5.37 Å for *para*-quinones. Our computation was performed on a Silicon Graphics IRIS CRIMSON/Elan workstation by use of *Builder* and *Discover* modules of Insight II (Biosym Technologies, versions 2.3.0 and 2.9.5, individually) for model building and energy minimization, respectively. The energies for all conformations were minimized with the consistent valence forcefield (CVFF) until the maximum derivative was less than 0.001 kcal/(mol Å).

Scheme 1



We prepared totally twenty four 1,5- or 1,7-azulenequinones according to the literature methods.[8,9] These azulenequinones contain an alkyl, halogen, alkoxyl, or amino substituent. To test their DNA cleaving capability, we irradiated azulenequinones (1000 μM) in a sodium phosphate buffer at pH 6.0 containing 10% EtOH and the supercoiled circular *φX174* RFI DNA (form I; 50 μM/base pair) with UV light (350 nm, 16-W) at room temperature under aerobic conditions for 2 h. Analytical results from gel electrophoresis on 1% agarose with ethidium bromide staining showed 12 among the 24 synthesized azulenequinones exhibited appealing DNA-cleaving activity. Single-strand scission of DNA took place by azulenequinones **1**–**12** to give the relaxed circular DNA (form II), as shown in Figure 1. The ratios of (form II)/(form I) ranged from 0.35–3.0. 3-Amino-1,5-azulenequinone **7** showed the greatest potency.

This new photo-induced cleaving process exhibited a pH-independent fashion for azulenequinone **7**: (form II)/(form I) = 3.1, 3.0, 2.7, and 2.5 at the pH 5.0, 6.0, 7.0, and 8.0, respectively. In control experiments, we removed molecular oxygen from the buffer by bubbling argon gas and removed singlet oxygen by adding sodium azide. The cleaving potency of **7** remained in both experiments. Accordingly we conclude that azulenequinones can cleave DNA under anaerobic conditions and may not involve singlet oxygen.

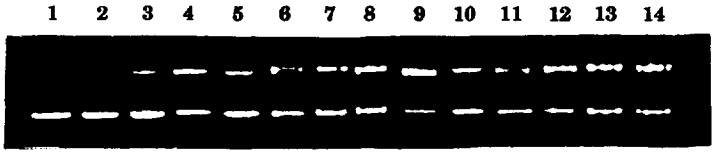
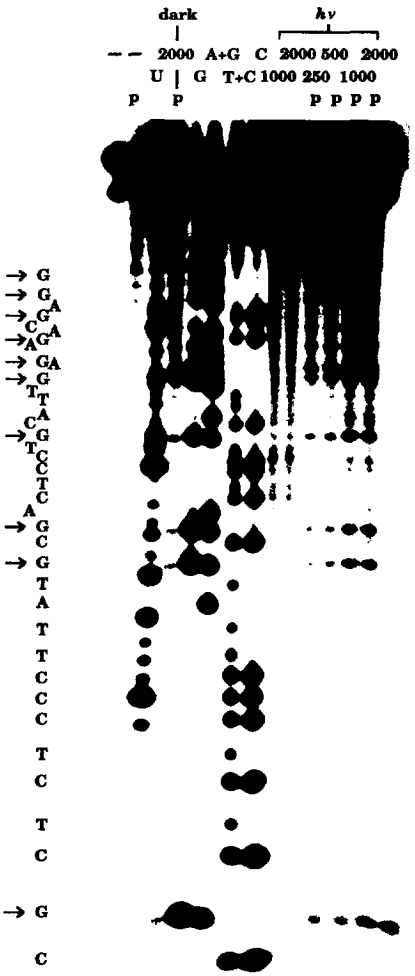
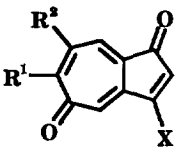
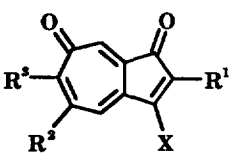


Figure 1. Single-strand cleavage of supercoiled circular *φX174* RFI DNA with an azulenequinone (1000 μM): Lane 1, DNA alone; Lane 2, **7** in the dark; Lane 3, **1**; Lane 4, **2**; Lane 5, **3**; Lane 6, **4**; Lane 7, **5**; Lane 8, **6**; Lane 9, **7**; Lane 10, **8**; Lane 11, **9**; Lane 12, **10**; Lane 13, **11**; Lane 14, **12**.





1. $R^1 = R^2 = H$, X = OMe
2. $R^1 = R^2 = H$, X = Cl
3. $R^1 = R^2 = H$, X = Br
4. $R^1 = H$, $R^2 = i$-Pr, X = Br
5. $R^1 = H$, $R^2 = i$-Pr, X = $NHCH_2CH_2OH$
6. $R^1 = i$-Pr, $R^2 = H$, X = NMe_2
7. $R^1 = R^2 = H$, X = NH(Ph-*p*-Me)



8. $R^1 = R^2 = R^3 = H$, X = Cl
9. $R^1 = R^3 = H$, $R^2 = i$-Pr, X = Br
10. R^1 = Me, $R^2 = i$-Pr, $R^3 = H$, X = Br
11. $R^1 = R^3 = H$, $R^2 = i$-Pr, X = NMe_2
12. $R^1 = R^2 = H$, $R^3 = i$-Pr, X = NMe_2

Figure 2. Autoradiogram of a denaturing polyacrylamide gel representing cleavage of 5'-^{32}P-labeled *SalI–SphI* fragment of pBR322 by photolysis of azulenequinone **7** (250–2000 μM): –, unincubated DNA; p: DNA fragments with piperidine treatment; U: DNase I cleavage ladder; G, A+G, T+C, C, nucleotide residues determined by Maxam–Gilbert chemical sequencing. The nucleotide sequence of the labeled strand of oligonuclotides is shown at the left. The photo-cleavage sites are indicated by "→".

Furthermore, we found that single-strand cleavage did not occur in the dark. Thus the UV light functioned as a "trigger"[10] to initiate the DNA single-strand scission by activating an azulenequinone.

To explore the DNA cleavage with site specificity, we carried out gel electrophoresis labeling experiments by using azulenequinone **7** (250–2000 μM). The 93 base pair *Sal*I–*Sph*I double-stranded DNA fragment of pBR322 with 5'-^{32}P-labeled on one strand was obtained enzymically.[11,12] Azulenequinone **7** in a sodium phosphate buffer at pH 6.0 containing 10% EtOH was then preincubated with the DNA fragment at 37 °C for 30 min. Irradiation of the sample with UV light (350 nm) followed by piperidine treatment at 95 °C for 30 min generated the pattern, as shown in Figure 2, on an autoradiogram of a 20% polyacrylamide/8 M urea gel. Use of piperidine as a base assisted the vision of the fragmentation pattern; however, it caused DNA scission in the dark to a limited extent. Our results indicate that azulenequinone **7** cleaved DNA dominantly at the guanine residue.

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