Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 6116–6118
Bioorganic & Medicinal Chemistry Letters

DNA cleavage by photolysis of aryl sulfoxides

Daniel J. Mayo, David P. Turner, Beth E. Zucconi and Allison H. Predecki*

Department of Chemistry, Shippensburg University, Shippensburg, PA 17257, USA

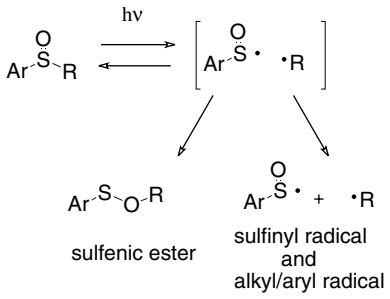
Received 27 July 2007; revised 10 September 2007; accepted 11 September 2007
Available online 15 September 2007

Abstract—Aryl sulfoxides have been identified as a class of organic compounds capable of inducing DNA cleavage in the presence of UV light. Phenyl sulfoxide and methyl phenyl sulfoxide were both shown to cleave pBR322 DNA at concentrations of 180 and 360 μM, respectively. Radical trapping studies indicate carbon-centered radicals are the active cleavage species.

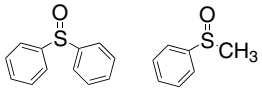
The identification of simple organic molecules capable of producing photogenerated free radicals that lead to DNA damage has been an interesting and important pursuit in the field of bioorganic chemistry.[1] DNA cleavage by carbon-centered radicals is one area of particular interest. Radical-initiated DNA cleavage is a significant mechanism in many cancer therapies[2] and chemical nucleases are useful tools in molecular biology.[3] These artificial nucleases generally are triggered in vivo to produce carbon-centered radicals. Enediynes were the first class of compounds discovered to undergo this type of mechanism.[4] Other types of molecules shown to produce carbon-centered radicals capable of DNA cleavage when triggered photochemically include organometallic complexes[5] and small molecule organics such as triazoles.[6] Herein we report photoinitiated carbon-centered radical DNA cleavage by a different class of organic compounds, aryl sulfoxides.

Fused ring aryl sulfoxides have recently been reported by Greer and co-workers to be DNA photocleaving agents with atomic oxygen [O(^{3}P)] as the proposed oxidant species.[7] In contrast, a primary photoprocess of non-fused ring aryl sulfoxides is homolytic cleavage of one of the C–S bonds forming a sulfinyl/alkyl or aryl radical pair (Scheme 1).[8] This α-cleavage is followed by the competing processes of free radical formation, recombination to form the parent sulfoxide, and formation a sulfenic ester. Sulfinyl radicals have not been observed to undergo hydrogen abstraction and therefore are unlikely to participate in DNA cleavage.[9] However, alkyl and aryl radicals have been shown previously to abstract a hydrogen from the sugar-phosphate backbone of DNA.[5b,10] Surprisingly, DNA cleavage studies with these aryl sulfoxides have never been reported (Scheme 2).

Two simple aryl sulfoxides[11] (**1** and **2**) were tested for DNA cleaving ability upon exposure to UV light using a plasmid relaxation assay in which DNA cleavage is indicated by alteration of supercoiled plasmid DNA (Form I) to relaxed circular DNA (Form II). In these experiments, the aryl sulfoxide, dissolved in DMSO,



Scheme 1. Photolysis mechanism of aryl sulfoxides.



1 **2**

Scheme 2. Aryl sulfoxides used in DNA cleavage.

Keywords: DNA cleavage; Chemical nuclease; Aryl sulfoxide.
* Corresponding author. Tel.: +1 717 477 1441; fax: +1 717 477 4048; e-mail: ahpred@ship.edu

0960-894X/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2007.09.049

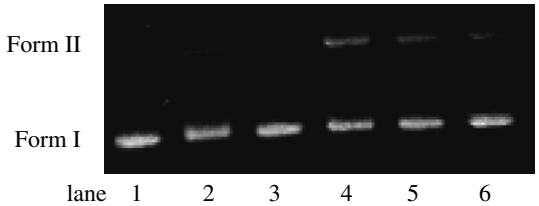
was mixed with pBR322 DNA in Tris–HCl buffer. The mixture was photolyzed for 10 min with a micro-photoreactor using a quartz Pen-Ray 5.5 W low pressure mercury lamp with a Vycor filter sleeve. The Vycor sleeve, which filters out UV light with wavelengths of 240 nm and below, was used to reduce damage to the DNA by the light while still allowing for photoactivation of the aryl sulfoxides.

Phenyl sulfoxide (**1**) was found to cleave DNA at concentrations of 180 μM and greater (6 molecules per base pair). Control experiments indicated that both the compound and UV light were required for DNA cleavage (Fig. 1). Methyl phenyl sulfoxide (**2**) was also found to cleave DNA under the same conditions at concentrations of 360 μM (Fig. 2).

Radical trapping experiments give insight into the mechanism of this DNA cleavage. Cysteine, when added to the reaction mixture, inhibited the cleavage (Fig. 3, lane 5). Cysteine is a general radical trap and therefore can trap any radicals generated from the aryl sulfoxides or the subsequent DNA radical.[13] Likewise, TEMPO, a carbon radical trap, also inhibited the DNA cleavage (Fig. 3, lane 6). TEMPO has been shown to trap induced carbon radicals on DNA in addition to small carbon radicals such as methyl radical.[14]

Hydroxyl radicals do not appear to play a role in the cleavage. The initial experiments were all done in the presence of DMSO, a hydroxyl radical trap, and DNA cleavage was observed in each instance (Figs. 1–3).[15] Additionally, performing the experiments in a different solvent, acetonitrile, did not increase the amount of cleavage observed (Fig. 4, lane 4).

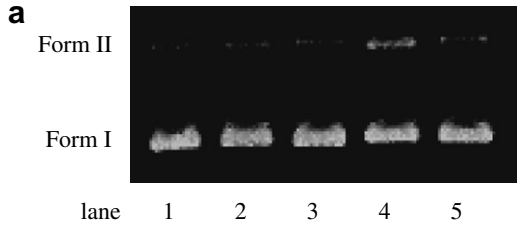
Cleavage by singlet molecular oxygen ($^1O_2$) also does not appear to be a viable mechanism. Adding cysteine to reactions in which singlet oxygen is generated has been shown to *increase* single-strand breaks in DNA.[16] When cysteine was added to the reaction of phenyl sulfoxide (**1**) with DNA, the cleavage was inhibited rather than increased (Fig. 3, lane 5). Ground state molecular oxygen ($^3O_2$), however, does appear to have a role in the mechanism. Excluding oxygen from the reaction by three freeze-pump-thaw cycles inhibited the DNA cleavage (Fig. 4, lane 5).



b

lane	% Form I	% Form II
1	99	1
2	96	4
3	100	0
4	71	29
5	83	17
6	93	7

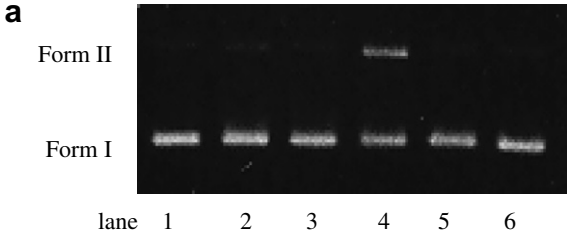
Figure 1. (a) Photoinduced cleavage of pBR322 DNA (30 μM/bp in 10% DMSO/10 mM Tris buffer, pH 8) by phenyl sulfoxide (**1**): lane 1, DNA alone, no irradiation; lane 2, DNA alone, irradiated; lane 3, DNA + phenyl sulfoxide (360 μM), no irradiation; lanes 4–6, DNA + -phenyl sulfoxide (360, 180, and 90 μM, respectively), irradiated. Reactions in lanes 2 and 4–6 were irradiated with Vycor filtered light from a 5.5 W low pressure mercury lamp for 10 min. (b) Quantitation of Form I and Form II DNA per lane.[12]



b

lane	% Form I	% Form II
1	100	0
2	99	1
3	99	1
4	83	17
5	97	3

Figure 2. (a) Photoinduced cleavage of pBR322 DNA (30 μM/bp in 10% DMSO/10 mM Tris buffer, pH 8) by methyl phenyl sulfoxide (**2**): lane 1, DNA alone, no irradiation; lane 2, DNA alone, irradiated; lane 3, DNA + methyl phenyl sulfoxide (360 μM), no irradiation; lanes 4 and 5, DNA + methyl phenyl sulfoxide (360 and 180 μM, respectively), irradiated. Reactions in lanes 2, 4, and 5 were irradiated with Vycor filtered light from a 5.5 W low pressure mercury lamp for 10 min. (b) Quantitation of Form I and Form II DNA per lane.[12]

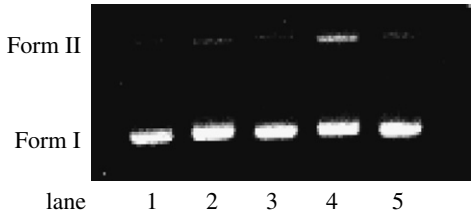


b

lane	% Form I	% Form II
1	97	3
2	94	6
3	97	3
4	67	33
5	95	5
6	99	1

Figure 3. (a) Inhibition by cysteine and TEMPO of photoinduced cleavage of pBR322 DNA (30 μM/bp in 10% DMSO/10 mM Tris buffer, pH 8) by phenyl sulfoxide (**1**): lane 1, DNA alone, no irradiation; lane 2, DNA alone, irradiated; lane 3, DNA + phenyl sulfoxide (360 μM), no irradiation; lane 4, DNA + phenyl sulfoxide (360 μM), irradiated; lane 5, DNA + phenyl sulfoxide (360 μM) + cysteine (36 mM), irradiated; lane 6, DNA + phenyl sulfoxide (360 μM) + TEMPO (36 mM), irradiated. Reactions in lanes 2 and 4–6 were irradiated with Vycor filtered light from a 5.5 W low pressure mercury lamp for 10 min. (b) Quantitation of Form I and Form II DNA per lane.[12]

a



b

lane	% Form I	% Form II
1	98	2
2	95	5
3	96	4
4	79	21
5	95	5

Figure 4. (a) Photoinduced cleavage of pBR322 DNA (30 μM/bp in 10% acetonitrile/10 mM Tris buffer, pH 8) by phenyl sulfoxide (**1**): lane 1, DNA alone, no irradiation; lane 2, DNA alone, irradiated; lane 3, DNA + phenyl sulfoxide (360 μM), no irradiation; lane 4, DNA + phenyl sulfoxide (360 μM), irradiated; lane 5, DNA + phenyl sulfoxide (360 μM), anaerobic, irradiated. Reactions in lanes 2, 4, and 5 were irradiated with Vycor filtered light from a 5.5 W low pressure mercury lamp for 10 min. (b) Quantitation of Form I and Form II DNA per lane.[12]

Greer and co-workers have reported that fused ring aryl sulfoxides cleave DNA via a photodeoxygenation reaction with atomic oxygen [O(^{3}P)] as the cleavage species.[7] In their experiments, DNA cleavage was observed under both aerobic and anaerobic conditions. With phenyl sulfoxide (**1**), DNA cleavage was not observed under anaerobic conditions (Fig. 4, lane 5). Therefore, a photodeoxygenation mechanism is not viable.

Based on these radical trapping studies, we conclude that the DNA cleavage by aryl sulfoxides is most likely the result of hydrogen abstraction from the sugar-phosphate backbone by a carbon-based radical (methyl or phenyl). As has been shown with organometallic complexes that cleave DNA via methyl radicals, this hydrogen abstraction is presumably followed by reaction of the induced DNA radical with oxygen leading to subsequent strand cleavage.[5b] Further experiments are needed to identify the mechanism definitively.

Acknowledgments

This work was financially supported by Shippensburg University Foundation Student Research Grants and Shippensburg University Center for Faculty Excellence in Scholarship and Teaching.

References and notes

1. For lead references see: Armitage, B. *Chem. Rev.* **1998**, *98*, 1171.
2. For lead references see: Povrik, L. F. *Curr. Opin. Onco., Endo., Meta. Invest. Drugs* **1999**, *1*, 370.
3. Hertzberg, R. P.; Dervan, P. B. *J. Am. Chem. Soc.* **1982**, *104*, 313.
4. For lead references see: Smith, A. D.; Nicolau, K. C. *J. Med. Chem.* **1996**, *39*, 2103.
5. (a) Riordan, C. G.; Wei, P. *J. Am. Chem. Soc.* **1994**, *116*, 2189; (b) Mohler, D. L.; Downs, J. R.; Hurley-Predecki, A. L.; Sallman, J. R.; Gannett, P. R.; Shi, X. *J. Org. Chem.* **2005**, *70*, 9093.
6. Wender, P. A.; Touami, S. F.; Alayrac, C.; Philipp, U. C. *J. Am. Chem. Soc.* **1996**, *118*, 6522.
7. Wauchope, O. R.; Shakya, S.; Sawwan, N.; Liebman, J. F.; Greer, A. *J. Sulf. Chem.* **2007**, *28*, 11.
8. Darmanyan, A. P.; Gregory, D. D.; Guo, Y.; Jenks, W. S. *J. Phys. Chem. A* **1997**, *101*, 6855.
9. Guo, Y.; Jenks, W. S. *J. Org. Chem.* **1997**, *62*, 857.
10. For lead references see: Pogozelski, W. K.; Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089.
11. Phenyl sulfoxide (96%) and methyl phenyl sulfoxide (97+%) were purchased from Sigma–Aldrich. Phenyl sulfoxide was recrystallized from ethanol before use. Methyl phenyl sulfoxide was used as received.
12. The bands in scanned gels were quantitated using NIH ImageJ software.
13. (a) Huston, P.; Espenson, J. H.; Bakac, A. *Inorg. Chem.* **1992**, *31*, 720; (b) Milligan, J. R.; Tran, N. Q.; Ly, A.; Ward, J. F. *Biochemistry* **2004**, *43*, 5102.
14. (a) Connolly, T. J.; Baldovi, M. V.; Mohtath, N.; Scaiano, J. C. *Tetrahedron Lett.* **1996**, *37*, 4919; (b) Hwang, J.-T.; Greenberg, M. M.; Fuchs, T.; Gates, K. S. *Biochemistry* **1999**, *38*, 14248.
15. Anbar, M.; Neta, P. *Int. J. Appl. Radiat. Isot.* **1967**, *18*, 493.
16. Devasagayam, T. P. A.; Di Mascio, P.; Kaiser, S.; Sies, H. *Biochim. Biophys. Acta* **1991**, *1088*, 409.