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Photocleavage of plasmid DNA by dibenzothiophene *S*-oxide under anaerobic conditions

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The ability of dibenzothiophene *S*-oxide (**1**) to photochemically induce strand breaks in plasmid DNA was studied under anaerobic conditions. DNA cleavage is monitored by the conversion of closed circular pUC19 DNA (form I) to the nicked (form II) and linear forms (form III) using densitometer digital imaging of ethidium stained gels. In buffered aqueous–acetonitrile (9:1) solutions the single-strand cleavage is efficient and does not require an alkaline reaction workup. Photodeoxygenation of **1** in buffered aqueous–acetonitrile (9:1) solutions containing benzene led to the production of phenol. The effect of solvent deuteration does not support the involvement of $^1O_2$ from a sulfoxide dimerization reaction nor a sensitized photooxygenation reaction. The results are interpreted in terms of a sulfoxide photodeoxygenation via oxygen atoms [O(^{3}P)] where oxidation of DNA can lead to single-strand breaks. Since the reaction of O(^{3}P) atoms with water itself is endoergic, we propose that hydroxyl radicals do not intervene in the DNA cleaving reaction.

Keywords: Photooxidation; Sulfoxide deoxygenation; Phototoxicity; DNA-cleavage; Polycyclic aromatic hydrocarbons

1. Introduction

It is unclear whether the chemistry of atomic oxygen [O(^{3}P)] has importance in biological oxidation processes. Few reactions can generate O(^{3}P) atoms under "mild" conditions in solution given the high energetics typically associated with its formation. However, advances have been made in photodeoxygenations of heterocyclic oxide compounds. It is likely that atomic O(^{3}P) is a key intermediate in some of these reactions [1–14]. Namely, UV irradiation of dibenzothiophene *S*-oxide (**1**) [1–4], 1,2-benzodiphenylene *S*-oxide (**2**) [9, 10], dibenzoselenophene *Se*-oxide (**3**) [13], pyridine *N*-oxide (**4**) [14] have been applied as probable sources of solution-phase atomic O(^{3}P). (The double bond formalism for SO and SeO is not intended to convey a pure double bond character). An understanding of the oxidation

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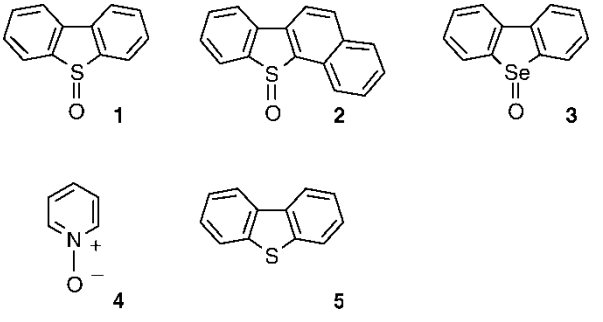
of organic molecules by atomic oxygen is known [15]. Atomic oxygen is a selective agent in the ground-state, O(^{3}P), and a non-selective agent in the excited-state, O(^{1}D) [15]. However, knowledge of a biological oxidation reaction involving O(^{3}P) is lacking, which stands in contrast to the knowledge of numerous other biological oxidants [16]. This led us to an examination of the photochemistry of **1**, where DNA cleavage is found to take place under anaerobic conditions. In 2003, the reduction of 3,4-dibenzyl-2,5-dimethylthiophene-*S*-oxide in combination with DNA on a glassy carbon electrode was reported to possibly lead to strand breaks in dsDNA [17]. No mechanistic inference can be made regarding the DNA cleavage.



2. Results and discussion

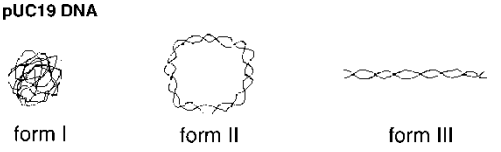
2.1 *Plasmid DNA nicking studies*

Sulfoxide **1** mediates the photocleavage of pUC19 DNA at wavelengths ca. 312–350 nm in buffered aqueous solutions (table 1). A schematic representation of pUC19 DNA is shown in Scheme 1 [supercoiled (form I), open circular (form II), linear (form III)]. We detected form II DNA in the greatest amounts when **1** was irradiated under aerobic or anaerobic conditions (entry 7 and 8, table 1). Form II DNA arises, but to a lesser extent in the absence of **1**, in the presence of light under anaerobic or aerobic conditions (cf. entry 1 with entry 2 and 3). Finally, form II DNA does not arise in presence of **1** or **5** in the dark (entry 4 and 5), nor does **5** induce strand breaks to a significant extent in the presence of light and molecular oxygen (entry 6).

Table 1. Photocleavage of plasmid DNA by dibenzothiophene *S*-oxide (**1**) and dibenzothiophene (**5**)[a,b].

Entry	Reaction	% Form I DNA[d]	S value
1	aerobic; DNA alone	80	0.2
2	aerobic; DNA + hν	77	0.3
3	aerobic[c]; DNA + hν	67	0.4
4	aerobic; DNA + **5**	82	0.2
5	aerobic; DNA + **1**	77	0.3
6	aerobic; DNA + **5** + hν	64	0.4
7	aerobic; DNA + **1** + hν	32	1.1
8	aerobic[c]; DNA + **1** + hν	25	1.4

[a]Photolysis of **1** or **5** (1 mM) was carried out in the presence of 312–350 nm light in 50 mM sodium phosphate buffer (pH 7.0) and 10% acetonitrile; [b]The percent error for form I DNA is ±10% and is the average of 2–4 runs; [c]Argon was bubbled into the reaction mixture for 5–10 minutes prior to irradiation; [d]Number of single strand breaks per plasmid molecule is determined based upon the equation: S = −ln (% form I DNA). S is the mean number of strand breaks calculated with S = −ln (% form I DNA); form III DNA not observed (ref. [18]).



SCHEME 1

A common photochemical DNA reaction by polycyclic aromatic hydrocarbons (PAH) involves energy transfer from the excited PAH to $^3O_2$ and the generation of $^1O_2$. Thus, we examined whether DNA cleavage may result from photosensitization giving rise to $^1O_2$. Reactions were conducted in the presence of a known $^1O_2$ sensitizer [19, 20], Al(III) phthalocyanine tetrasulfonic acid chloride (entry 1–3, table 2). A relatively small effect arises with deuterated solvents in the DNA photocleavage in the presence of the sensitizer (cf. entry 2 and 3). The solvent effect does not suggest $^1O_2$ as a key intermediate in the DNA damage. The experiments in table 2 were conducted because $^1O_2$ might arise from the presence of trace $^3O_2$ present even after Ar purging. A dimerization reaction [11, 21] to generate $^1O_2$ also represents an alternative to unimolecular SO fragmentation of **1** (equations (1)–(3)). However, a source of $^1O_2$ either via route (equations (2) or (3)) would not explain the subsequent oxidation chemistry that we observe (benzene oxidation to phenol, described below).

$$\underset{\mathbf{1}}{\mathrm{DBTO}} \xrightarrow{h\nu} \mathrm{O(^3P)} + \underset{\mathbf{5}}{\mathrm{DBT}} \tag{1}$$

$$\text{sensitizer or } \mathbf{1} + {}^3O_2 \xrightarrow{h\nu} \text{sensitizer or } \mathbf{1} + {}^1O_2 \tag{2}$$

$$2\,R_2SO \xrightarrow{h\nu} \underset{\text{dimer}}{R_2SO{-}OSR_2} \longrightarrow 2\,R_2S + {}^1O_2 \tag{3}$$

2.2 *Benzene hydroxylation to afford phenol*

The reactivity of the intermediate produced in the photodeoxygenation of **1** was probed with benzene trapping under the same solvent conditions used in the DNA nicking experiments. Photodeoxygenation of **1** in the buffer solution containing benzene (~0.01 M) demonstrate a hydroxylation yielding phenol (equation (4)) according to GC/MS. We have previously attributed an electrophilic character to $O(^3P)$ in acetonitrile based on trapping agents, such as benzene, *p*-substituted styrenes, p,p'-disubstituted diaryl sulfides, 2-methylbutane, and

Table 2. Effect of deuterated solvents on the photocleavage of plasmid DNA by dibenzothiophene *S*-oxide **1**[a–c].

Entry	Reaction	% Form I DNA
1	aerobic; D_2O/CD_3CN; DNA + sens	77
2	aerobic; D_2O/CD_3CN; DNA + sens + hν	66
3	aerobic; H_2O/CH_3CN; DNA + sens + hν	74
4	aerobic; D_2O/CD_3CN; DNA + **1** + hν	16
5	aerobic[c]; D_2O/CD_3CN; DNA + **1** + hν	17

[a]Photolysis of **1** (1 mM) was carried out in the presence of 312–350 nm light in 50 mM sodium phosphate buffer (pH 7.0) and 10% suitably labeled acetonitrile; [b]The experimental error percent form I DNA ±10% based on the average of 2–4 runs; [c]Argon was flushed through the reaction mixture for 5–10 minutes prior to irradiation. The sensitizer is Al(III) phthalocyanine tetrasulfonic acid chloride.

chloride ion [9, 10]. The subsequent oxidation of these chemical traps yielded phenol, epoxides, aldehydes, sulfoxides, alcohols, and hypochlorite ion. A mechanism was suggested that involved $O(^3P)$ based on the regioselectivity and substituent effects of the product distributions.

$$C_6H_6 + \mathbf{1} \xrightarrow{h\nu} C_6H_5OH + \mathbf{5} \tag{4}$$

2.3 *DNA cleaving mechanism*

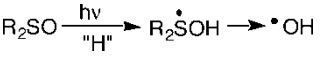
Since light and **1** were required for DNA cleavage and since under identical conditions added benzene is converted to phenol a DNA cleaving mechanism is possible where atomic oxygen is the reactive agent (equations (1)–(4)). Here, the SO bond of **1** can be photochemically fragmented. Several alternative mechanisms may be considered against the possible chemistry of $O(^3P)$. Ground state **1** is not an oxidant, but in the excited state the SO bond breaks leading to compound **5**. The SO bond dissociation energy (BDE) of a typical sulfoxide, such as that of Ph_2SO is approximately 95 kcal/mol. In contrast, fused aromatic sulfoxides **1** and **2** possess BDEs that are reduced ~75 kcal/mol since the sulfur atoms are enclosed within an aromatic ring [22–24]. Thus, **1** and **2** are special in the sense that many sulfoxides are not expected to photochemically fragment due to BDEs that often reside above their corresponding singlet excited state energies. The idea of a sulfinyl radical intermediate in the DNA cleaving process seems unlikely since the sulfinyl radicals are poor oxygen-transfer agents and also possess high SO BDEs (~101 kcal/mol) (equation (5)) [25, 26]. Furthermore, the oxidation of olefins to epoxides by sulfinyl radicals has been reported to be endothermic by ~20 kcal/mol (equation (6)). An excited-state bimolecular S-O deoxygenation process via a bridge with DNA is possible (equation (7)). However, we do not suggest bimolecular S-O deoxygenation of **1** to be a key process. The reason is that Jenks *et al.* reported that the quantum yield for S-O deoxygenation of **1** varies little in solvents of different nucleophilicity (e.g., alkanes, freons, alcohols), which implies unimolecular S-O bond cleavage [1, 2]. Formation of hydroxyl radicals is an endoergic process and thus not expected to participate in the DNA cleaving chemistry (equation (8)). Hydrogen abstraction via a Norrish type II reaction with a sulfoxide is unprecedented in spite of research effort to induce such a process (equation (9)) [27]. Photolytic pathways to ring expansion have been recognized in some systems, but may not predominate for **1**. For example, the photolysis of 9-cyanoacridine *N*-oxide **6** gives the ring-expanded product **7** (equation (10)) rather than an N_{ar}-oxide deoxygenation reaction as a result of lower degree of aromaticity of acridines relative to pyridines [28–31]. The photolysis of dibenzoselenophene *Se*-oxide **3** leads to two ring-expanded products according to NMR [13]. At present, we do not have reason to suggest that ring-expanded dibenzothiophene sulfoxide plays a role in the DNA cleavage. It is tempting to suggest that a reaction between triplet oxygen atoms and DNA involves hydrogen atom abstraction from the DNA sugar-phosphate backbone or the DNA bases, which would lead to strand cleavage [32]. The hydrogen abstraction reaction of atomic oxygen with DNA could lead to the formation of hydroxyl radical and amplify the reactivity given the subsequent reaction between hydroxyl radical and DNA.

$$R_2SO \xrightarrow{h\nu} R^{\bullet} + RSO^{\bullet} \tag{5}$$

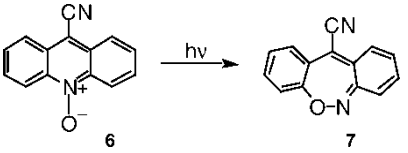
$$RSO^{\bullet} + H_2C{=}CH_2 \longrightarrow RS^{\bullet} + H_2C\overset{O}{\frown}CH_2 \tag{6}$$

$$R_2SO + DNA \xrightarrow{h\nu} {}^{1,3}R_2S^{*}{-}O{-}DNA \tag{7}$$

$$O(^3P) + H_2O \longrightarrow 2\ {}^{\bullet}OH \tag{8}$$



(9)



(10)

3. Conclusion

Sulfoxide **1** is discussed as a new type of DNA photocleaving agent. The formation of form II DNA demonstrates that strand cleavage takes place, but does not identify the reactive agent. We propose an $O(^3P)$-mediated DNA photocleavage. In other chemistry, many papers have focused on DNA cleavage with oxidants, such as hydroxyl radicals and singlet oxygen. This work suggests the first example of a biologically relevant $O(^3P)$ reaction. The work also points to a possible $O(^3P)$ toxicity that has not been considered in the past.

4. Experimental section

Reagents and solvents were obtained commercially [dibenzothiophene **5**, benzene, phenol, sodium phosphate, agarose, TAE buffer, ethidium bromide, glycerol, glycerol gel loading solution, Tris, bromophenol blue, biphenyl, aluminum(III) phthalocyanine tetrasulfonic acid chloride, D_2O, acetonitrile, and acetonitrile-d_3] and were used as received. pUC19 DNA was purchased from Boehringer Mannheim and used as received. Compound **1** was prepared by a reaction of **5** with *m*-chloroperoxybenzoic acid. Gas chromatographic data were acquired on one of two gas chromatographs, a Hewlett-Packard GC/MS instrument consisting of a 5890 series GC and a 5988A series mass selective detector, or on a Shimadzu-17A auto-sampler capillary gas chromatograph equipped with a flame ionization detector. An HP-5 capillary column was used in the GC/MS instruments. The GC temperature was ramped using the following program: initial oven temperature 80 °C for 10 min., 15 °C/min. to 150 °C and held for 6 min., 7 °C/min. to 200 °C and held for 9 min., 30 °C/min. to 250 °C and held for 15 min. The injector and detector temperatures were held at 250 °C. NMR measurements were carried out on a Bruker (400 ^{1}H MHz) spectrometer. Photolyses were conducted with a 75-W Xenon PTI Model L-201 Arc lamp focused on a tunable monochromator to obtain monochromatic light over the range 280–400 nm (linear dispersion equal to ca. ±12 nm). Some photolyses were conducted with a FisherBiotech TLC lamp that contained two 312 nm tubes (115 V). A typical experiment contained 1 mM **1**, 40 ng pUC19, in 50-μL Ar-saturated 50 mM sodium phosphate buffer (pH 7.0) with 10% acetonitrile.

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