Hydroxyl Radical is the Active Species in Photochemical DNA Strand Scission by Bis(peroxo)vanadium(V) Phenanthroline

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Bis(peroxo)vanadium(V) complexes are widely investigated as anticancer agents. They exert their antitumor and cytotoxic effects through inhibition of tyrosine phosphatases and DNA cleavage, respectively. The latter process remains poorly understood. The mechanism of DNA cleavage by NH₄[(phen)V(O)(η²-O₂)₂] (phen = 1,10-phenanthroline) was investigated. Kinetic studies on DNA cleavage revealed that the complex is a single-strand nicking agent with no specificity. EPR experiments using 2,2,6,6-tetramethyl-4-piperidone (TMP) and 5,5′-dimethyl-1-pyrroline-N-oxide (DMPO) as spin-traps for singlet oxygen and hydroxyl radical, respectively, implicated hydroxyl radical production upon photodecomposition of bis(peroxo)vanadium(V). This was corroborated by benzoate inhibition of DNA strand scission and stoichiometric oxidation of 2-propanol to acetone upon irradiation of bis(peroxo)-vanadium(V) phenanthroline. High-resolution polyacrylamide gel analysis of the vanadium cleavage reaction and [Fe²⁺EDTA]²⁻/H₂O₂ resulted in comigration of “ladder” pattern bands, which superimposed when both reactions were run on the same lane. These findings identify hydroxyl radical produced from the photooxidation of the peroxo ligand on vanadium as the active species in DNA cleavage.

Introduction

Vanadium is a trace metal with a dietary intake requirement of 10–60 μg day⁻¹. Due to its minimal requirement in higher animals, vanadium is not defined as an essential element. However, vanadium complexes, particularly, vanadates and more recently peroxovanadium compounds, have been implicated in many biological processes and therapeutic applications, as insulin-mimetics and antitumor agents. Vanadium complexes have been shown to reduce hyperglycemia in diabetic rats and NIDDM patients. Furthermore, vanadium compounds exhibit antitumor activity by inhibiting growth of numerous malignant cell lines including L1210 leukemia, Hela cells, and human ovary carcinoma, by induction of cell-cycle arrest and/or cytotoxic effects. Their anti-proliferative effects are induced by the ability of peroxovanadium complexes to cleave DNA and initiate lipoperoxidation. Production of reactive oxygen species (ROS) upon intracellular reduction or photoactivation has been alluded to. However, the mechanisms of ROS generation and DNA cleavage remain uncharacterized.

Hiort et al. reported first on the photochemical cleavage of DNA by NH₄[(Phen)V(O)(η²-O₂)₂] (Phen = 1,10-phenanthroline), BpVphen (Chart 1), which remains the most active vanadium complex toward DNA. Subsequently, Kwong et
activity when D2O was used as solvent, and the EPR spin-inhibited DNA cleavage, there was enhancement in cleavage was based on the following observations: sodium azide release of singlet oxygen (1O2).

To address these outstanding questions, we examined a poor oxidant and does not lead to complete strand breakage.5 To address these outstanding questions, we examined in detail DNA photocleavage by BpVphen. In this report, we present kinetics of supercoiled DNA cleavage, radical scavenger studies, EPR spin-trap experiments, and time-resolved spectroscopy of singlet oxygen luminescence decay, as well as high-resolution polyacrylamide gel analysis of radiolabeled DNA oligos. Results from these investigations point to the identity of the ROS that is responsible for strand scission in the BpVphen system.

Experimental Section

Reagents. Materials were purchased from the following suppliers: TRIS, sodium benzoate, formic acid, sodium acetate, EDTA, ammonium persulfate, low melting agarose, glycerol, urea, acrylamide, bisacrylamide, 2-propanol, H2O2 (30% w/v), Fisher Scientific; ammonium vanadate (NH4VO3), Matheson Co.; 1,10-phenanthroline monohydrate, 5,5′-dimethyl-1-pyrroline-N-oxide (DMPO), 2,2,6,6-tetramethyl-4-piperidone (TMP), Aldrich. NH4[V(O)(O2)2(bpy)] (bpy = 2,2′-bipyridine);7 the authors proposed reduction of V(V) to a peroxovanadium(III) concomitant with release of singlet oxygen (1O2). Evidence of 1O2 involvement was up for speculation. Furthermore, singlet oxygen is considered a poor oxidant and does not lead to complete strand breakage.5

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DNA Substrates. pSP64 plasmid DNA was a gift from Dr. Carla Sam et al., examined the DNA photocleavage activities of 15 peroxovanadium complexes focusing in detail on [V(O)(O2)2(bpy)]− (bpy = 2,2′-bipyridine);7 the authors proposed reduction of V(V) to a peroxovanadium(III) concomitant with release of singlet oxygen (1O2). Evidence of 1O2 involvement was up for speculation. Furthermore, singlet oxygen is considered a poor oxidant and does not lead to complete strand breakage.5 To address these outstanding questions, we examined in detail DNA photocleavage by BpVphen. In this report, we present kinetics of supercoiled DNA cleavage, radical scavenger studies, EPR spin-trap experiments, and time-resolved spectroscopy of singlet oxygen luminescence decay, as well as high-resolution polyacrylamide gel analysis of radiolabeled DNA oligos. Results from these investigations point to the identity of the ROS that is responsible for strand scission in the BpVphen system.

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DNA Substrates. pSP64 plasmid DNA was a gift from Dr. Carla Koehler’s laboratory. Plasmid was overexpressed in DH10b and isolated using a modified alkaline lysis method. Supercoiled form was isolated prior to each experiment. The purification procedure includes running the prep DNA on a 1% low melting agarose gel at 100 V for 2 h and extracting it using a Qiagen kit. Herring testes DNA (sodium salt) was purchased from Sigma Chemical Co. and dialyzed against a 0.5 M, pH 8.09 EDTA using 0.025 μm pore size MF-Millipore membranes to remove divalent ions.9 The concentration of DNA, expressed in base pairs, was calculated spectrophotometrically using an ε260 nm value of 6.7 × 104 M−1 cm−1.10

Linear DNA of 2.8 kbp was obtained from a double restriction digest of pSP64 plasmid using EcoRI and PvuII restriction endonucleases. The restriction fragment was gel purified on a 1% low melting agarose and extracted using Qiagen kit, followed by treatment with alkaline phosphatase.

A DNA fragment of 135bp was generated from a PCR amplification reaction from yeast genomic DNA. Short single-stranded oligonucleotides T-20mer (5′-TTTTTTTTTTTTTTTTTTTTT-3′) and AG-20mer (5′-AAAGAGAGGAAGGGAAGGGG-3′) were purchased commercially from BioSource. These oligos were PAGE purified. Oligos were loaded on 20% (37.5:1) polyacrylamide/7 M urea sequencing gel and electrophoresed at 30 W for 13 h. Bands were cut out and oligos were isolated by electroelution.

5′-32P end-labeling of 135 bp fragments and oligos were achieved by incubating DNA substrate with [γ-32P]ATP and T4 polynucleotide kinase for 30 min at 37 °C. The enzyme was deactivated by incubating 100 °C for 2 min. The end-labeled product was eluted off G-25 spin column with deionized H2O or 50 mM Tris buffer at pH 7.4.

DNA cleavage for pSP64 plasmid and linear form was analyzed on 1.5% agarose gel (containing 0.5 μg/mL ethidium bromide) which was electrophoresed at 120 V for 2 h followed by visualization on a UV transilluminator. Quantification of DNA cleavage was achieved by comparing the intensity of bands to intensity of mass ladder bands. All measurements were done using ImageQuant 5.2 program. A correction factor of 1.5 was multiplied to the values obtained for supercoiled DNA to adjust for differential staining.11

Cleavage reactions for 5′-32P end-labeled 135 bp fragments and 20-mer oligos were visualized on 12% and 24% (19:1) polyacrylamide/7 M urea sequencing gels that were electrophoresed at 100 W or 2800 V for 3 and 6 h, respectively. Gels were then transferred to Whatman paper, dried, and exposed on phosphomager screen. Gels were then scanned with phosphomager.

Photolysis. Samples, contained in Fisherbrand flint glass test tubes (6 × 50 mm o.d.) or 3-mL quartz cell were irradiated using a 100 W mercury lamp (λmax ~ 350 nm). A UG-1 glass filter and a copper sulfate solution filter were used to cut off the visible (425–675 nm) and IR regions, respectively. In experiments where the samples were electrophoresed after irradiation, reactions were quenched by adding loading dyes containing either 0.09% bromophenol blue, 0.09% xylene cyanol FF, 60% glycerol, 60 mM EDTA or 80% formamide, 10 mM EDTA at pH 8, 1 mg/mL xylene cyanol FF.

Kinetics. All kinetics studies on DNA cleavage were carried out with supercoiled DNA at 20 °C in 50 mM pH 7.5 Tris-HCl. The amount of DNA used in a 10 μL reaction volume was ca. 500

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ng. Time-dependent studies were done for a range of BpVphen concentrations (0–1.0 mM). Initial rates were determined by measuring the decrease in supercoiled form DNA. Rate constants were then plotted against [BpVphen].

To measure photocleavage as a function of [BpVphen], reactions containing ca. 300 ng of supercoiled form DNA and various BpVphen concentrations (0–3.0 mM) in 10 µL of reaction volume were irradiated for 2 h. The amount of cleavage was measured and plotted against [BpVphen].

Irradiation of Linear DNA in the Presence of Radical Scavengers. In a total of 10 µL of reaction volume, 200 ng of linear DNA and 1 mM BpVphen in the presence or absence of 100 mM azide or benzoate was irradiated for 5 h. Percent cleavage was measured and compared for reactions with and without scavengers.

NMR Experiments. In all NMR experiments, a 1:1 mixture of D$_2$O and 0.1 M, pH 7.4 sodium phosphate buffer ([NaH$_2$PO$_4$]/[Na$_2$HPO$_4$]) was used as the solvent. 1H NMR spectra were collected on a Bruker ARX-400 MHz NMR or Bruker ARX-500 MHz NMR. 31V NMR spectra were collected on a Bruker ARX-500 MHz NMR, and VOCl$_3$ was used as an external reference.

Oxygen Evolution Experiments. Oxygen evolution experiments were conducted with YSI Model 5300 biological oxygen monitor equipped with a Clark oxygen electrode and a chart recorder. In all oxygen evolution experiments, air saturated 50 mM pH 7.4 Tris-HCl was used as the solvent. The concentrations of bpvphen used were in a range of 0.075 to 3.0 mM in 10 µL of 0.5 M EDTA pH 8. Maxam–Gilbert G+A sequencing reaction and [Fe$^2$EDTA]$^{2-}$/H$_2$O$_2$ reaction. In the DNase I reaction, 1 U of DNase I was incubated with 0.4 µg of radiolabeled 20-mer oligo for 5 min at 37 °C. The reaction was quenched with 0.4 µL of 0.5 M EDTA pH 8. Maxam–Gilbert G+A sequencing reaction and [Fe$^2$EDTA]$^{2-}$ reaction were followed as described in Current Protocols in Molecular Biology.6,12 and Pogozelski et al.,18 respectively.

Results

Photochemical Cleavage of pSP64 Plasmid. A typical cleavage profile for BpVphen is shown in Figure 1A. Supercropped plasmid (form I), upon irradiation into the BpVphen LMCT band at $\lambda \approx 350$ nm, was converted initially to open circular (form II), which at longer irradiation times was converted to linear (form III) and small fragments. This pattern of discrete cleavage steps depicts a single-strand process. Supercropped form requires one single cleavage event to unravel its structure to open-circular form.19,20 Eventual conversion to small fragments requires longer irradiation time to produce breaks on both strands of the DNA substrate.


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These small fragments are characterized by the smears on the gel. The regular smears indicate lack of sequence specificity. In the absence of light, the rate of conversion from supercoiled to open-circular form was 60 times slower than with photolysis (data not shown) showing that DNA strand scission is accelerated by irradiation and the contribution from any thermal reaction is negligible. Moreover, irradiation of BpVphen in the presence of supercoiled plasmid under anaerobic conditions resulted in formation of open-circular DNA, excluding the possibility that exogenous oxygen plays a role in DNA cleavage.

Even though the cleavage process is random, information on the kinetics of cleavage can be obtained from the first step in the conversion of supercoiled to open circular form. The rate of supercoiled plasmid DNA disappearance showed a hyperbolic dependence on [BpVphen] (Figure 1B). The kinetic saturation at high [BpVphen] is due to reaching the power limit of the photolysis lamp since a plot of supercoiled DNA fraction versus [BpVphen] taken at a fixed irradiation time (2 h) was exponential (Figure 1C).

To identify ROS generated upon irradiation of BpVphen, different radical scavengers were screened with linear DNA (Figure 1D). After 5 h of irradiation, reactions containing DNA and BpVphen resulted in 70% cleavage (Figure 1D). With either azide (lane 4) or benzoate (lane 5), cleavage was inhibited, resulting in no or minimal (c.a. 10%) cleavage, respectively. On the basis of these results, singlet oxygen and hydroxyl radical are implicated. While benzoate is a recognized scavenger of hydroxyl radical but not singlet oxygen, azide quenches singlet oxygen and has been shown to also react with hydroxyl radical.

Photodecomposition of BpVphen. $^{51}$V NMR spectra of the photodecomposition of BpVphen as a function of irradiation time were acquired (Figure S1, Supporting Information). At longer irradiation times (> 2 h), BpVphen decomposed to simple vanadates, monomer ($\text{H}_2\text{VO}_4^-$) as the major product, dimer ($\text{H}_2\text{V}_2\text{O}_7^{2-}$), and cyclic tetramer ($\text{V}_4\text{O}_{12}^{2-}$), which are characterized by their chemical shifts ($\delta$) at $-561$, $-572$, and $-577$, respectively. The photodecomposition of BpVphen was complete within 2 h of irradiation, as the fractional integrations of decomposition products remained constant. During the course of irradiation, minor peaks at $\delta$ $-675$, $-660$, and $-621$ were observed intermediates (Figure S1). The peaks at $-675$ and $-621$ ppm could be attributed

to [VO\textsubscript{2}(\eta\textsuperscript{2}-O\textsubscript{2})\textsuperscript{-}]\textsuperscript{2-} and [V(OH)(\eta\textsuperscript{2}-O\textsubscript{2})]\textsuperscript{-24} respectively. The peak at -660 ppm may arise from a peroxovanadium dimeric species.\textsuperscript{25}

**Oxygen Evolution and Singlet Oxygen Detection Experiments.** Irradiation of degassed solution of BpVphen resulted in evolution of molecular oxygen, which was quantified using an oxygen electrode. Table 1 lists the concentrations of BpVphen used in each experiment, irradiation time, and the yield of molecular oxygen. Evolved oxygen corresponded to approximately 0.5 equiv/vanadium, which supports O\textsubscript{2} production from dimeric vanadium peroxo species.

To test whether the evolved O\textsubscript{2} is singlet, we attempted to measure the characteristic luminescence decay of singlet oxygen at 1270 nm with time-resolved spectroscopy.\textsuperscript{13,14} No \textsuperscript{1}O\textsubscript{2} was detected. Since a highly sensitive germanium photodiode detector was used and a positive control for \textsuperscript{1}O\textsubscript{2} was successfully carried out, it is reasonable to conclude that the evolved oxygen from BpVphen is triplet.

**Oxidation of 2-Propanol to Acetone.** To probe for hydroxyl radical generation upon irradiation of BpVphen, the oxidation of 2-propanol to acetone was examined. Hydroxyl radical reacts with 2-propanol to yield 2-propanol radical via C(2)–H abstraction, according to eq 1.\textsuperscript{26} The 2-propanol radical reacts with a number of metal ions including VO\textsuperscript{2+} (eq 2) to produce acetone.\textsuperscript{27,28}

\[
\text{(CH}_3\text{)}_2\text{CHOH} + \text{HO}^\cdot \rightarrow \text{(CH}_3\text{)}_2\text{COH} + \text{H}_2\text{O} \quad (1)
\]

\[
\text{(CH}_3\text{)}_2\text{COH} + \text{VO}^{2+} + \text{H}^+ \rightarrow \text{VO}^{2+} + \text{(CH}_3\text{)}_2\text{CO} + \text{H}_2\text{O} \quad (2)
\]

In our experiments, a solution containing 1.0 mM BpVphen was irradiated in the presence of 40 mM 2-propanol, and acetone production was quantified by \textsuperscript{1}H NMR. Since the end products of the photodecomposition of BpVphen are simple vanادات (H\textsubscript{2}VO\textsubscript{4}\textsuperscript{-}, H\textsubscript{2}V\textsubscript{2}O\textsubscript{7}\textsuperscript{-}, and V\textsubscript{6}O\textsubscript{12}\textsuperscript{2-}), the phenanthroline ligand must dissociate. Thus, the amount of acetone produced from the reaction was quantified by comparing the integrations of free phenanthroline peaks (\(\delta = 7.68, 7.85, 8.38, \text{and} 8.93\)) and the integration of the acetone peak (\(\delta = 2.10 \text{ ppm}\)), as illustrated in Figure S2. A stoichiometric amount of acetone (0.99 ± 0.06 mM) relative to vanadium was obtained in three duplicate runs. On the other hand, carrying out the 2-propanol oxidation reaction in the presence of 40 mM sodium benzoate, a known scavenger of hydroxyl radical, resulted in 0.53 mM acetone, a 46% reduction. Singlet oxygen does not oxidize 2-propanol; this fact was confirmed in a control experiment in which 40 mM 2-propanol was irradiated in the presence of methylene blue, a singlet oxygen sensitizer.\textsuperscript{29} The control reaction did not produce detectable acetone.

**Electron Paramagnetic Resonance (EPR) Spin-Trap Experiments.** Additional support for hydroxyl radical production from photoactivation of BpVphen was provided by EPR spin-trap experiments. 5,5’-Dimethyl-1-pyrroline-N-oxide (DMPO) and 2,2,6,6-tetramethyl-1-piperidone (TMP)
were used as spin-traps for hydroxyl radical and $^1$O$_2$, respectively. Irradiation of BpVphen in the presence of DMPO clearly enhanced the characteristic signal for the DMPO/$\cdot$OH adduct (a 1:2:2:1 spectrum with a hyperfine splitting $a_N = a_H = 14.9$ G) (Figure 2). Photolysis of a solution of BpVphen and DMPO in the presence of sodium benzoate resulted in a ca. 70% reduction in the signal intensity (Figure 2E). In contrast, photolysis of BpVphen in the presence of TMP did not enhance the characteristic 1:1:1 EPR spectrum of 2,2,6,6-tetramethyl-4-piperidone-N-oxyl, which is formed upon reaction with $^1$O$_2$ (Figure S3).

Cleavage of 5'-32P End-Labeled DNA Fragments. Cleavage of radiolabeled DNA offers a direct and sensitive approach to identifying cleavage products. A sequencing gel of photocleavage of 5'-32P end-labeled 135bp DNA fragment with 2 mM BpVphen showed production of small fragments of various sizes forming a “ladder” pattern, Figure 3, indicative of random cleavage. The bands within each lane display similar intensity, demonstrating lack of base selectivity. Irrespective of the sequence or length of the DNA substrate, the same “ladder” pattern was observed for either 5'-labeled T-20mer or 5'-labeled AG-20mer oligos (Figure 3B,C). The advantage of using short oligos is the ability to visualize cleavage pattern at the nucleotide level. For both oligos, cleavage was observed at every base regardless of the type or position. This result demonstrates lack of base specificity. At each nucleotide, doubling of bands denotes multiple cleavage pathways resulting in fragments of different termini. Cleavage results were indistinguishable whether Tris or phosphate was used as the solvent buffer. Tris has been suggested to react with hydroxyl radical when employed at concentrations $>0.3$ M. However, at the concentration level utilized in our studies (50 mM), Tris does not interfere with the efficiency of DNA cleavage.

To identify the different cleavage termini, the BpVphen cleavage reaction was run alongside a DNase reaction and a Maxam–Gilbert sequencing reaction that are known to produce 3'-hydroxyl and 3'-phosphate terminus, respectively, Figure 4A. No comigration was observed between the bands obtained from the vanadium reaction (lane 2) and those from DNase (lane 1). In contrast, comigration was observed between the first band of the cleavage reaction (lane 2) and

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Figure 3. (A) Phosphoimage of polyacrylamide gel (10%) of cleavage of 5'-32P end-labeled 135 bp DNA fragment. Cleavage was observed as a function of time with 2 mM BpVphen. Phosphoimages are shown of high-resolution polyacrylamide (24%) gel of cleavage reactions with (B) T-20mer oligo and (C) AG-20mer oligo. Key: (B) lanes 1 and 2, controls without BpVphen, photolyzed for 0 min and 1 h, respectively, lanes 3–7, with increasing irradiation time; (C) lanes 1 and 2, controls without BpVphen, photolyzed for 0 min and 2 h, respectively, lanes 3–7, with increasing irradiation time.

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those of the Maxam–Gilbert reaction (lane 3). The tapering off in the bands is due to differences in salt concentrations and running buffer. Hence, when the two reactions, BpVphen and Maxam–Gilbert G+A, are run on the same lane, the corresponding bands comigrate, doubling their intensity (lane 4 of Figure 4A).

To identify the second band of the doublet pattern, we ran the BpVphen cleavage reaction alongside the known [Fe(II)EDTA]^{2-} cleavage reaction (Figure 4B).\(^{18,33}\) The latter is known to produce hydroxyl radical, which attacks DNA to give fragments ending in both 3'-phosphate and 3'-phosphoglycolate group.\(^{18,33-37}\) Both reactions featured identical cleavage profile (lanes 2 and 3), and when they were run on the same lane (lane 4), all bands superimposed. This finding clearly points to hydroxyl radical as the oxidant in peroxovanadium DNA-cleaving reactions.

Attempts to identify base damage did not meet with much success, and the results were inconclusive. For example, standard piperidine treatment of photolyzed samples with BpVphen and radiolabeled DNA gave increasing intensity of cleaved bands. However, this observation was not informative with regards to alkali-labile lesions since controls with piperidine alone and no BpVphen resulted in DNA


cleavage. Additionally, a primer extension assay\(^{53}\) as well as a MALDI-TOF (matrix-assisted laser desorption/ionization time-of-flight) mass spectrometric analysis were not interpretable, most likely due to the lack of site specificity of the BpVphen system.

Discussion

The experimental findings described here provide a compelling case that hydroxyl radical is the ROS produced upon photolysis of BpVphen and it is the oxidant responsible for DNA cleavage in this system. The following results are cited in support of hydroxyl radical production: (1) inhibition of DNA cleavage by sodium benzoate, which is widely used as a probe for hydroxyl radical;\(^{33}\) (2) positive detection of the characteristic signal for the DMPO/OH adduct in EPR spin-trap experiments; (3) oxidation of 2-propanol to acetone upon irradiation of BpVphen; (4) DNA cleavage as a single-strand scission process displays no specificity; (5) high-resolution polyacrylamide gel analysis of photocleavage with BpVphen being identical to the profile with [Fe\(^{III}\)EDTA]\(^{2-}\), which is known to generate hydroxyl radical to effect oxidative DNA cleavage.\(^{18,33-37}\)

Although other ROS had not been fully probed relative to hydroxyl radical, they were excluded because they are considered poor agents for breaking DNA. Superoxide and hydrogen peroxide were examined by others as agents for strand scission of DNA and were found ineffective.\(^{38,39}\) However, their role is to facilitate the formation of hydroxyl radical via Fenton-like reactions with transition metals. As will be discussed below, the vanadium complex studied in this report undergoes similar reactions to generate hydroxyl radical.

Singlet oxygen was explored because Kwong et al. implicated it as the oxidant in DNA cleavage by the analogous bis(peroxo)vanadium(V) bipyridine, BpVbpy.\(^{1}\) \(O_2^{-}\) oxidizes bases, preferentially at guanine, to form abasic sites, which require further treatment to induce strand scission.\(^5\) Experiments conducted in this study to identify base modifications (piperidine treatment, primer extension assay, and mass spectrometry) yielded inconclusive results. Nevertheless, EPR spin-trap experiments and time-resolved luminescence spectroscopy did not detect singlet oxygen. The utility of an oxygen selective electrode demonstrated that photolysis of BpVphen evolved a 0.5 equiv/vanadium of \(O_2\), which we concluded must be triplet oxygen since the characteristic luminescence of \(O_2\) at 1270 nm was not observed with a radical mechanism in which hydroxyl radical is produced was put forth.\(^{28,40}\) We observed similar products for the photochemical decomposition of BpVphen; simple vanadates were the final products with evolution of 0.5 equiv molecular oxygen. Parallel to these literature reports, we propose in Scheme S1 a viable pathway for the production of hydroxyl radical. Photooxidation of the peroxo ligand of BpVphen results in cleavage of the \(V-O_peroxo\) bond; the transient is protonated to yield hydroperoxyl radical, which reacts with mono(peroxo)vanadium to give hydrogen peroxide, oxygen, and vanadyl. The latter reacts with \(H_2O_2\) by Fenton-like chemistry to form hydroxyl radical.\(^{41}\)

The conversion of plasmid DNA to open circular and from open circular to linear and small fragments (Figure 1A) demonstrated that cleavage by BpVphen is a single strand process. Cleavage was observed regardless of the length or sequence of DNA substrates, indicating lack of specificity. The cleavage profiles included smears and “ladder” patterns on agarose and high-resolution polyacrylamide gels, respectively (Figures 1A and 3). The bands on the “ladder” were equal in intensity demonstrating same probability of attack on any nucleotide. Hydroxyl radical has no charge, is freely diffusible, and, thus cleaves DNA indiscriminately.

Hydroxyl radical-mediated DNA strand scission has been thoroughly investigated.\(^{5,17,33,36}\) The radical abstracts hydrogen from the deoxyribose ring, leading to strand scission to produce fragments that terminate in a 5'-phosphate, 3'-phosphate, or 3'-phosphoglycolate group. If the DNA is labeled at the 5'-end, then only 3'-termini are visualized on high-resolution polyacrylamide gel. Indeed, when DNA cleavage was done with short oligos of 20 bp, a doubling of bands was observed at each nucleotide (Figure 3B,C). These bands comigrated and superimposed with bands produced by Fenton–Udenfriend reaction, which is known to produce hydroxyl radical (Figure 4B). Therefore, all the experiments described in this study put together support the proposal that hydroxyl radical is generated from the photoactivation of the BpVphen and it is the agent responsible for cleavage of DNA.

Conclusion

There has been much speculation on whether the cleavage agent of BpVphen is a transient vanadium species or reactive oxygen radical. We have reported herein the mechanism of DNA cleavage by NH\(_4\)[(phen)V(O)(\(\eta^2\)-O\(_2\))]\(^{2-}\). This report supports the notion of hydroxyl radical as the active agent. The radical is generated by Fenton-like reactions upon photooxidation of the peroxo ligands of the complex. Results from EPR spin-trap experiments and comparison of BpVphen cleavage profile with that of [Fe(II)EDTA]\(^{2-}\)/\(H_2O_2\) incriminate hydroxyl radical as the culprit from the two distinct sources.

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Supporting Information Available: Figures of a stack plot of $^{51}V$ NMR spectra for the photodecomposition of BpVphen, an $^1H$ NMR spectrum of 2-propanol oxidation, EPR spectra of the spin-trap TMP, and a suggested mechanism accounting for the formation of HO$^\bullet$ from BpVphen (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.