DNA-Photocleavage Activities of Vanadium(V)−Peroxo Complexes†

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Reagents which can cleave nucleic acids under mild conditions have potential applications as DNA- and RNA-sequencing reagents as well as antitumor and antiviral drugs. Recently, a number of redox-active transition-metal-based nucleases have been shown to cleave DNA and/or RNA with photoactivation[1] or in the presence of cofactors.[2,3] Phenanthrenequinonediimine complexes of Rh(III) are known to recognize and photocleave DNA in a sequence-specific manner;[2,3] and Fe(II)−EDTA,[4] its methidium-tethered complex,[2] and bis(1,10-phenanthroline)Cu[I] utilize hydrogen peroxide to generate hydroxyl radicals to effect the oxidative cleavage of DNA and RNA.[5,6] Thus, it is conceivable that transition-metal complexes with peroxide ligands are likely to be effective nucleases as well. Several vanadium(V)−peroxo complexes have been shown to exhibit antileukemic activities.[7] More recently, Hiort et al. reported the DNA-photocleavage activities of two diperoxovanadium(V) complexes with 1,10-phenanthroline and 4,7-dimethyl-1,10-phenanthroline as ancillary ligands.[8] In this work, we have examined the DNA-photocleavage activities of 15 vanadium(V)−peroxo complexes. A mechanism for their photocleavage activities, which involves singlet oxygen produced from the photolysis of these complexes, observed for the *first* time in vanadium(V), is proposed.

Using a plasmid DNA relaxation assay,[9] the photocleavage activities of these vanadium(V)−peroxo complexes were measured. The quantitative DNA-photocleavage activities, together with the absorption spectral parameters, of the complexes are summarized in Table 1.

From among the various active complexes, the $[VO(O_2)_2(bpy)]^-$ anion was chosen for a detailed mechanistic study. Since the (irreversible) oxidation potentials (*vs* SCE) of these complexes at pH 7 range from 0.7−1.0 V,[10] a direct oxidation of DNA by these complexes is thermodynamically unlikely. A reactive oxygen species, such as the hydroxyl radical and singlet oxygen, is more likely to be the species responsible for the DNA scission. Working on this hypothesis, we conducted experiments in the presence of different specific scavengers to probe the contributions of the respective potential reactive oxygen species in the observed DNA-photocleavage. To probe for hydroxyl radicals, sodium benzoate and ammonium formate[11] were used as scavengers whereas for singlet oxygen, sodium azide[12] was employed. Only sodium azide was found to quench the photocleavage activity of $[VO(O_2)_2(bpy)]^-$ significantly and in a concentration-dependent manner. The involvement of singlet oxygen is implicated.

The lifetime of singlet oxygen in D_2O (58 μs) is about 30-fold that in H_2O (2 μs).[13] Thus, the observed enhancement in cleavage activity when D_2O was used as the solvent reinforces

Table 1. DNA-Photocleavage Activities of Vanadium(V)−Peroxo Complexes Irradiated at 365 nm in an Aqueous Medium at pH 7.5

complex at 1 mM concn	cleavage activity (% conversion)[a]	λ_{max}, nm (ϵ, M^{-1} cm^{-1})	ref[b]
$NH_4[VO(O_2)_2(bpy)]\cdot 4H_2O$	50	354 (598)	23
$Na[VO(O_2)_2(bpy)]\cdot 5H_2O$	63	354 (598)	23
$NH_4[VO(O_2)_2(Me_2bpy)]\cdot 2H_2O$	55	344 (617)	23
$NH_4[VO(O_2)_2(phen)]\cdot 2H_2O$	99	327 (1390)	23
$NH_4[VO(O_2)_2(Me_2phen)]\cdot 2H_2O$	95	328 (1500)	24
$NH_4[VO(O_2)_2(Me_4phen)]\cdot 5H_2O$	99	331 (2640)	24
$NH_4[VO(O_2)_2(NO_2phen)]\cdot 2H_2O$	99	326 (6340)	this work[c]
$K_3[VO(O_2)_2(ox)]\cdot 2H_2O$ (at 5 mM)	6.2	328 (681)	23
$K[VO(O_2)_2(H_2O)_2]\cdot 2H_2O$	9.0	317 (720)	this work[c]
$K_2[VO(O_2)(cit)]_2\cdot 2H_2O$	25	413 (340)	25
$K_2[VO(O_2)(nta)]\cdot 2H_2O$	3.5	428 (411)	26
$H[VO(O_2)(pic)_2]$	30	434 (311)	24
$K[VO(O_2)(dipic)(H_2O)]\cdot 2H_2O$	9.1	430 (456)	27
$[VO(O_2)(terpy)(H_2O)]ClO_4\cdot H_2O$	23[d]	458 (373)[e]	this work[c]
$NH_4[VO(O_2)(ida)]\cdot 2H_2O$	9.2	420 (363)	28

[a] The DNA-cleavage activity is expressed in terms of the percentage conversion of the supercoiled or covalently-closed circular (ccc) conformer to the open-circular (oc) and/or linearized conformers of the plasmid DNA (pBluescript). The % conversion (*ca.* ± 5%) is calculated using

$$\% \text{ Conversion} = 1 - \frac{[\text{ccc}]_t}{[\text{ccc}]_0}$$

where $[ccc]_0$ is its concentration in the control and $[ccc]_t$ is its concentration in sample after incubating at ambient temperature for time *t*. [b] References for syntheses and structures of the complexes. [c] These complexes were prepared by us. Their syntheses and structures will be detailed in a separate publication. [d] The aqueous assay medium contained 7.5% acetonitrile required for easy dissolution of the complex. [e] The spectrum was obtained in acetonitrile.

† Abbreviations: bpy = 2,2′-bipyridine; Me_2bpy = 4,4′-bipyridine; phen = 1,10-phenanthroline; Me_2phen = 4,7-dimethyl-1,10-phenanthroline; Me_4phen = 3,4,7,8-tetramethyl-1,10-phenanthroline; NO_2phen = 5-nitro-1,10-phenanthroline; ox = oxalate; cit = citrate; nta = nitrilotriacetate; pic = pyridine-2-carboxylate; dipic = pyridine-2,6-dicarboxylate; terpy = terpyridine; ida = iminodiacetate, pipes = piperazine-*N*,*N*′-bis(2-ethanesulfonic acid); BPHA = *N*-benzoyl-*N*-phenylhydroxylamine.

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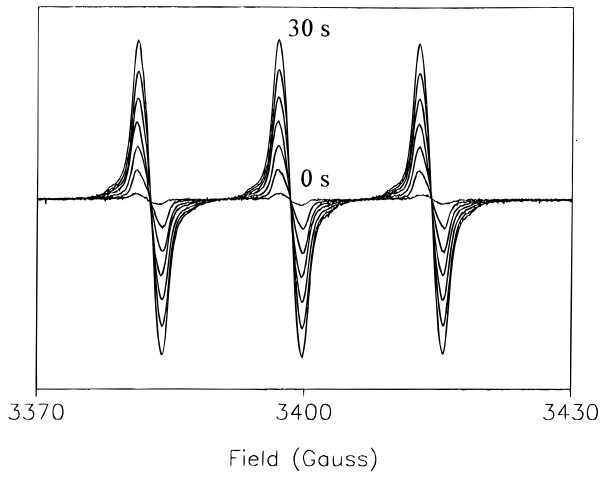


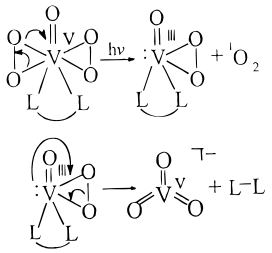
Figure 1. EPR spectra of a solution containing 10 mM of the $[VO(O_2)_2(bpy)]^-$ anion and 300 mM 2,2,6,6-tetramethyl-4-piperidone (TMP) at pH 9.0 as a function of photoirradiation time. The samples were irradiated for 5 s intervals for a total of 30 s using a 500 W high-pressure Hg lamp (Oriel) filtered through a water bath and a 260−380 nm bandpass filter. Spectra were collected at room temperature using the following parameters: scan rate, 50 G/min; time constant, 32 ms; microwave frequency, 9.514 GHz; microwave power, 5 mW; modulation amplitude, 2.5 G; modulation frequency, 100 kHz. The figure shows nitroxide formation corresponding to the presence of singlet oxygen after 0, 5, 10, 15, 20, 25, and 30 s of irradiation. The presence of a small amount of nitroxide before irradiation is the nitroxide impurity present in the commercially available TMP.

the notion that singlet oxygen is involved in the DNA-photocleavage process. Furthermore, no diminution of DNA-photocleavage activity was observed when experiments were carried out under quasi-anaerobic conditions, indicating that dissolved oxygen is not the source of singlet oxygen and photosensitization is not responsible for the observed activity.

Photolysis−EPR spin-trapping experiments were conducted to provide further support that singlet oxygen is the reactive species. Using 2,2,6,6-tetramethyl-4-piperidone (TMP) as a spin trap, we were able to detect the formation of 2,2,6,6-tetramethyl-4-piperidone-*N*-oxyl (a 1:1:1 spectrum with $g = 2.0059$ and a hyperfine coupling constant of 16.15 G).[14] The intensity of this nitroxide EPR signal was found to increase with the photolysis time of the $[VO(O_2)_2(bpy)]^-$ anion, as shown in Figure 1, and was quenched in proportion to the concentration of sodium azide present (data not shown). These data therefore confirm the production of singlet oxygen in the photolysis of the $[VO(O_2)_2(bpy)]^-$ complex.

Since the peroxide-to-singlet oxygen conversion is a net two-electron oxidation, these electrons are presumably taken up by the vanadium, resulting in the formation of V(IV) or V(III). The EPR experiments, however, did not reveal the presence of any oxovanadium(IV) species, which would give a characteristic 8-line spectrum with a *g* value of *ca.* 1.98.[15] The electronic spectrum of the reaction mixture also showed no sign of either a V(III) or V(IV) species. On the other hand, vanadium(V) was shown to be the product oxidation state using a spectrophotometric assay based on a V(V)-selective chelate formation reaction, V(V)−BPHA.[16] A ligand-centered oxidation was thus indicated. Finally, using ^{51}V NMR spectroscopy,[17] the spectrum

Scheme 1



of the photolysis product was shown to be identical to the spectrum obtained by dissolving authentic NH_4VO_3 in the same buffer solution, therefore identifying the metavanadate(V) anion, VO_3^-, as the vanadium product.

On the basis of the results obtained, we propose the mechanism shown in Scheme 1 for the photolysis ($\lambda_{irrad} = 365$ nm) of the $[VO(O_2)_2(bpy)]^-$ anion, at pH 7.5. In this proposed mechanism, the two electrons from the photooxidation of the peroxo ligand are placed on the metal in the form of a V(III) intermediate. Since no explicit attempts have been made to detect any V(III) species formed during the course of photolysis, we cannot rule out the possibility that the two electrons are in the bipyridine ring system. We also do not know at which step the ancillary ligand L−L comes off from the complex.

Recent studies have shown that exposure of DNA to $^1O_2$ leads to base damage and strand breaks, both of which occur specifically at the guanine residues.[18−20] While the chemistry of the $^1O_2$-mediated modification of the guanine base has been quite well-addressed,[21,22] the mechanism of DNA strand breaks without alkali- or base-treatment is still unclear.[18] Finally, outstanding questions remain in this study. (1) Is the DNA cleavage sequence-specific or site-specific? (2) What are the DNA damage products? Are they consistent with those $^1O_2$-mediated damage products (i.e., guanine-specific oxidation products such as 8-oxo-7,8-dihydro-2′-deoxyguanosine and 4,8-dihydro-4-hydroxy-8-oxo-2′-deoxyguanosine) found in type II (singlet oxygen) photosensitization reactions?[21,22] Further study to address these issues and other mechanistic details is currently underway.

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