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Site-Selective DNA Photocleavage Involving Unusual Photoinitiated Tautomerization of Chiral Tridentate Vanadyl(V) Complexes Derived from *N*-Salicylidene α-Amino Acids

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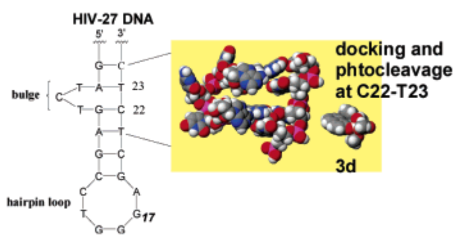
ABSTRACT



The titled vanadyl(V) complexes serve as efficient reagents for cleaving supercoiled plasmid DNA by photoinitiation. Complex 3d, derived from 2-hydroxy-1-naphthaldehyde and L-phenylalanine, exhibits a unique wedge feature, inducing a site-selective photocleavage at the C22-T23 of the bulge backbone for a HIV-27 DNA system at 0.1−5 μM. Transient absorption experiments for 3d indicate the involvement of LMCT with concomitant tautomerization, leading to an *o*-quinone-methide V-bound hydroxyl species responsible for the cleavage profiles.

Reagents that can achieve cleavage of nucleic acids in physiological aqueous media are potentially useful in DNA and RNA sequencing or as antitumor and antiviral drugs.[1] Several redox-active transition-metal-based nucleases have been shown to cleave DNA and/or RNA via photoinitiation[2] or in the presence of cofactors.[3] When combined with H_2O_2, a number of transition metal complexes[4] can facilitate oxidative[5] or phosphate diester[6] cleavage of DNA/RNA by

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incipient (or metal-bound) hydroxyl radicals or peroxo species. Photocleavage of DNA by vanadium(V) bisperoxo complexes (vanadates) is noteworthy in view of the singlet oxygen-generation mechanism involved.[7] It should be noted that the bisperoxo vanadate complex provides its own freely dissociated singlet oxygen in a stoichiometric manner for the cleavage process. Therefore, development of a real vanadyl(V)-associated DNA photocleaver without bearing any peroxo units or resorting to any co-oxidants remains to be explored.

As part of an ongoing program on the uses of chiral vanadyl(IV/V)[8] and oxometallic[9] complexes in new catalytic reactions, we have recently found that vanadyl(V) complexes may function as photoinitiators in acrylate and methacrylate polymerizations.[9] In addition, despite in-depth studies on the uses of vanadyl(IV)-peroxide adducts and di-peroxo-vanadates in DNA photocleavages have been documented, the direct action of vanadyl(V) complexes and their chiral entities to such event has not been explored. We herein describe our findings on their profiles in DNA cleavages when exposed to light without any co-oxidants.

A preliminary study in our laboratory has shown that *N*-salicylidene-based vanadyl(V) complexes **3** are capable of cleaving supercoiled plasmid DNA (form I), Coli phage ΦX-174, in aqueous DMSO (10−50%) into small fragments by photoinitiation with a 15 W UV table (λ > 315 nm) for 15 min with concentrations of the complexes as low as 10 μM.[10] Notably, these complexes **3** were obtained by recrystallization of the corresponding vanadyl(IV) complexes from MeOH. The original vanadyl(IV) complexes were oxidized spontaneously to vanadyl(V) species **3** during recrystallization, as evidenced by X-ray crystallographic analyses. Elemental analyses of these vanadyl(V) complexes **3** strongly indicate that they tend to exist as either monomers, as shown in Figure 1, or dimers with μ-oxo bridge (i.e., V(O)−O−V(O)).

In marked contrast to the common diperoxo-vanadate complex-mediated photocleavage mentioned above, the photocleavage processes with our chiral vanadyl(V) complexes **3** proceeded smoothly in the absence of H_2O_2. More importantly, the parent vanadyl(IV) complexes are completely incompetent under similar reaction conditions. The results strongly indicate that a conceptually very different mechanism is operating in the photocleavage process and is unique to the vanadyl(V) species.

To gain further insight into the working elements associated with our vanadyl(V) complexes in the cleavage process,

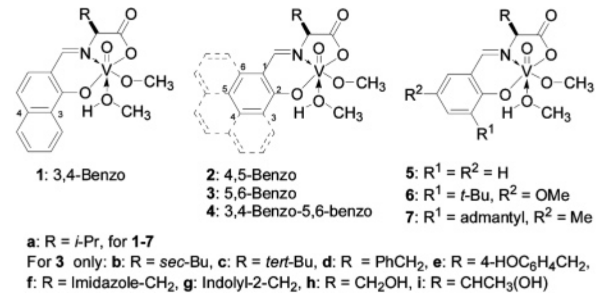

Figure 1. Vanadyl(V) complexes for photocleavage experiments.

a series of vanadyl(V) complexes **1**−**7** were synthesized with varying (1) extents of steric and/or electronic (conjugation) attributes in the template and (2) steric and chirality factors of the pendant group (R) in the α-amino carboxylate moiety, Figure 1.

Through kinetic trace experiments, it was found that *N*-salicylidene vanadyl(V) complex **5a** (R^1 = R^2 = H) derived from L-valine (R = *i*-Pr) tends to induce faster photocleavage of ΦX-174 than the corresponding naphthalidene **3a** (by a factor of 3), leading to the complete disappearance of supercoiled form I in 2 min (Figure S1, Supporting Information).[11a,12] As compared to 3,5-disubstituted cases (**6a** and **7a**), the essential breakdown of ΦX-174 into small DNA pieces was attained by employing the less sterically demanding and more water-soluble parent **5a** (compare lane 2 with lanes 3 and 4, Figure S1). Complexes derived from 5,6-benzo-*N*-salicylidenes **3a** and **4a** (lanes 5 and 8, Figure S1) were more efficient in mediating the photocleavage than the 3,4- and 4,5-benzo analogues (**1a** and **2a**, lanes 6 and 7, Figure S1), indicating the significant role of the template (naphthalene) orientation relative to the pendant chiral group (i.e., *i*-propyl) during the cleavage event (vide infra).

The pendant chiral group (R) in the α-amino carboxylate moiety of the vanadyl(V) complex also plays a discernible role in the rate of photocleavage. Notably, the pendant chiral group effect is more pronounced in the 5,6-benzo-*N*-salicylidene-based systems **3**. The photocleavage rate decreases with increase in the steric bulk of the pendant alkyl group. Among the three different alkyl pendant groups examined, the sterically less demanding valine-based vanadyl(V) complex **3a** (R = *i*-Pr) was the most reactive mediator (i.e., *i*-Pr-**3a** > *sec*-Bu-**3b** ∼ *t*-Bu-**3c**; compare lanes 2, 4, and 5 in Figure 2) in the test photocleavage. In contrast, serine-based (R = CH_2OH, lane 6) system **3h** is less reactive than threonine (R = $CHCH_3OH$, lane 7) system **3i**. On the other hand, among four different aromatic pendant groups examined, the noncoordinating phenylalanine-based vanadyl(V) complex **3d** (R = $PhCH_2$) was the most efficient mediator (compare lanes 8, 9, 11, and 12 in Figure 2).[11b]

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(9) Unpublished results from this laboratory.

(10) Concentration for supercoiled plasmid DNA phage is 3.7 nM (per strand).

(11) See: (a) Figure S1, (b) Figure S2 and S2′, (c) Figure S3, (d) Figure S4, (e) Figure S5, (f) Figure S6, (g) Figure S7, (h) Figure S8, (i) Figure S9, and (j) Figure 10 in Supporting Information for details.

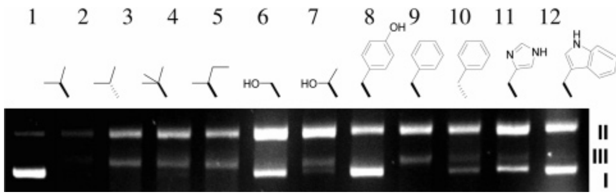

Figure 2. Photocleavages of ΦX-174 (3.7 nM per strand) with varying pendant R groups of vanadyl(V) complexes **3** (5 mM) for 6 min in 10 mM phosphate buffer (pH 6.9). Photocleavage products were analyzed by agarose gel electrophoresis. Lane 1, control.

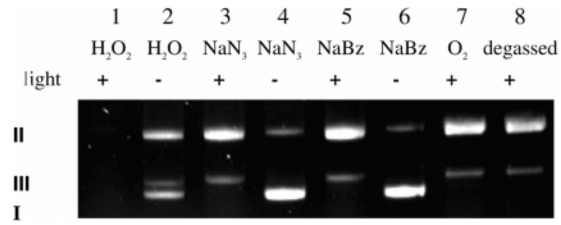

Figure 3. Effects of co-oxidants and quenchers on the photocleavages of ΦX-174 (3.7 nM per strand) by complex **3d** (5 mM) for 6 min. The additives, including H_2O_2, NaN_3, and NaBz, are all at 50 mM concentration.

In addition, the L-form valine and phenylalanine-based vanadyl complexes (**3a** and **3d**) are more reactive than the corresponding D-form antipodes (*ent*-**3a** and **3d′**). Complete disappearance of form I (supercoiled) along with further cleavage of form III (linear) was observed within 6 min in both L-form systems (lanes 2 and 9, Figure 2). However, small amounts of form I still remained for the test photocleavage mediated by the corresponding enantiomeric complexes (lanes 3 and 10, Figure 2). Furthermore, for a reduced photocleavage time of 4 min, the density integration ratios for (form II + form III)/form I amount to 2:1 for the photocleavage mediated by **3a** and *ent*-**3a**,[11b] respectively, suggesting that there exists a chirality match and mismatch effect between the vanadyl(V) complex **3a** and the supercoiled DNA duplex.

To clarify whether a photosensitization mechanism is involved for the observed cleavage activity with these vanadyl(V) complexes, we carried out a series of control experiments with **3d** in the presence of H_2O_2, sodium azide (a singlet oxygen quencher), or sodium benzoate (NaBz, a hydroxyl radical quencher).[13] Remarkably, the cleavage process continued to operate anaerobically (lane 8, Figure 3) and in the presence of 10-fold excess of NaN_3 (lane 3) or NaBz (lane 5), thus excluding the participation of any singlet oxygen or free radical mechanism associated with the vanadyl(V) complex-mediated photocleavage under the reaction conditions. Interestingly, partial cleavage of Coli phage ΦX-174 to form II (nicked) and form III (linear) DNAs were observed with the addition of aqueous H_2O_2 (50 mM) and **3d** (5 mM) in a 10:1 ratio (lane 2, Figure 3).[11c] A further significant increase in cleavage extent (with complete disappearance of forms I−III) resulted when irradiation was performed (lane 1). The extensive photocleavage in the latter case (lane 1) had to do with the peroxovanadate formation through the action of H_2O_2 as the co-oxidant. Under this circumstance, singlet oxygen may be generated in situ and responsible for the DNA cleavage.[7]

To further explore the utility of the vanadyl(V) complex **3d** as a potential agent of site-selective DNA photocleavage, HIV-27 DNA was tested with varying catalyst concentrations in the absence of any co-oxidant.[14,15] Preferential cleavages at the guanine residues of the HIV-27 were observed with higher loading (≥100 μM) of **3d**.[11d] On the other hand, selective cleavages at thymine bases, including T8, T14, T21, and T23, were observed in the presence of 2.9 mM (29-fold) of H_2O_2.[11e] In addition, random photocleavage was observed with **3d** in the presence of ≥290-fold excess of H_2O_2 (≥29 mM), presumably due to the generation of free $^1O_2$.[11e] In marked contrast, highly site-selective photocleavage at the C22−T23 site of the bulge backbone was attained under 100 times reduction in concentration (1−5 μM) for **3d** (Figure 4b) in the absence of H_2O_2, despite the fact that both the bulge and hairpin loop sites contain the same 5′-T/C−CT-3′ sequence.[16] In addition, the cleavage profile remains the same down to 0.1 μM for **3d**.[11f] Notably, the enantiomeric complex of **3d** (i.e., **3d′**) was less efficient by a factor of 5. Besides photocleavage at C22−T23, guanine cleavages at the loop (site 15−17) and bulge (site 9) were also observed with **3d′**.[11d] Photocleavages were found to be less selective with other vanadyl(V) complexes **3**.

The preferential photocleavage at the C22−T23 site of the bulge backbone by **3d** may be rationalized in terms of the unique wedge feature associated with vanadyl(V) complex **3d** as evidenced by the X-ray crystal structure.[17] Complex **3d** recrystallized from MeOH exhibits a distorted octahedral geometry with two extra ligands from MeOH

(12) First band corresponds to nicked (circular, one strand cut) form II and the second band corresponds to linear (two strands cut) form III.

(13) (a) Muller, T.; Schuckelt, R.; Jaenicke, L. *Arch. Toxicol.* **1991**, *65*, 20. (b) Foote, C. S. In *Singlet Oxygen*; Wasserman, H. H., Murray, R. W., Eds.; Academic: New York, 1979; pp 139−171.

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(15) HIV-27 DNA substrate is a 27mer with the following sequence 5′-GCAGATCTGA−GCCTGGGAGC−TCTCTGC-3′, which forms a double-stranded DNA structure with a three-base bulge and a six-base single-stranded loop. This DNA sequence is modified from the sequence of the RNA hairpin from the trans activation response element (TAR-RNA). (a) Carter, P. J.; Breiner, K. M.; Thorp, H. H. *Biochemistry* **1998**, *37*, 13736. (b) Varmus, H. *Genes Dev.* **1988**, *2*, 1055.

(16) Selective oxidations of guanine base(s) on bulge by Ni(II)−Schiff base complexes in the presence of oxidants have been demonstrated by Burrows and co-workers: (a) Shih, H.-C.; Kassahun, H.; Burrows, C. J.; Rokita, S. E. *Biochemistry* **1999**, *38*, 15034. (b) Chen, X.; Burrows, C. J.; Rokita, S. E. *J. Am. Chem. Soc.* **1992**, *114*, 322. For salicylidene Schiff base Cu(II) complexes as photosensitizers for in situ singlet oxygen generation: (c) Reddy, P.; Santra, B. K.; Nethaji, M. *J. Inorg. Biochem.* **2004**, *98*, 37. (d) Dhar, S.; Senapati, D.; Das, P. K.; Chattopadhyay, P.; Nethaji, M.; Charkravarty, A. R. *J. Am. Chem. Soc.* **2003**, *125*, 12118.

(17) (a) See Supporting Information for details. (b) For an X-ray structure of a vanadyl(V) complex from salicylaldehyde and L-alanine, see: Nakajima, K.; Kojima, M.; Toriumi, K.; Saito, K.; Fujita, J. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 760.

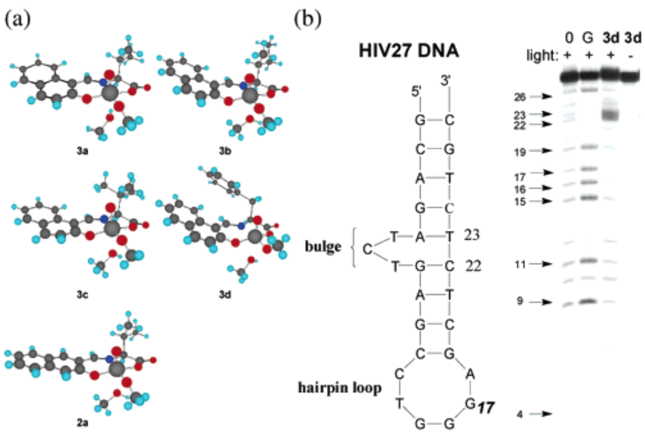

Figure 4. (a) Chem-3D presentation for the X-ray structure of **3a**[8a]−**d** and **2a**. (b) Selectivity profile of HIV-27 DNA (5 μM) photocleavage by vanadyl(V) complex **3d** (5 μM). Cleavage products followed by piperidine treatment were analyzed on a 20% denaturing polyacrylamide gel (7 M urea). Lane 1, DNA control with photoirradiation; lane 2, Maxam−Gilbert G marker; lane 3, DNA and **3d** with photoirradiation; lane 4, DNA and **3d** without photoirradiation.

(Figure 4a).[11g] One methanol is positioned trans to the V═O. The other one is covalently linked and is trans to the imine nitrogen, which may be responsible for the unique photochemical behavior associated with **3d** (vide infra). In addition, the V═O bond is syn to the benzyl group in the chiral template.[18] The $\pi-\pi$ stacking effect between the naphthalene template in **3d** and the aromatic floor provided by the G9−C22 pair and the additional $\pi-\pi$ interaction between the pendant benzyl group in **3d** and the A5-T23 pair serve to dock the V═O unit in close proximity to the C22−T23 spinal core.

To understand the partitioning cleavage behaviors under varying complex concentrations, molecular simulation of the theoretical UV spectrum for **3d** in aqueous DMSO was carried out.[11h] Fluorescence lifetime measurement of the blue emitting complex **3d** (λ_{em} = 450 nm) in 50% aqueous DMSO shows one distinctive decaying species, with lifetime (τ) corresponding 0.51 ns.[11i] We believe that the LMCT activation of the vanadyl (V═O) unit, followed by H atom transfer from the methanol ligand or from ligated H_2O, results in the blue emitting (λ_{em} = 450 nm), V-bound, multiple hydroxyl species **A**, Figure 5a. Alternatively, vanadyl(V) complex **3d** may function as a nonemitting UV absorber with initial photoinduced tautomerization of the template followed by similar intramolecular H atom transfer to form *o*-quinone methide-type, V-bound, multiple hydroxyl species **B**.[19] Subsequent nonradiative vibrational relaxation of **B** to

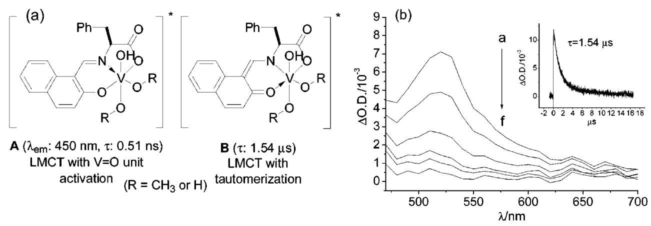

Figure 5. (a) Proposed species upon photoactivation of **3d**. (b) Temporal evolution of transient absorption spectra for complex **3d** (absorbance ∼ 1.2 at 400 nm) in aqueous DMSO (50%) at a pump−probe delay time of (a) 0, (b) 0.9, (c) 1.8, (d) 3.5, (e) 5.5, (f) 11 μs. Inset: The decay profile of the transient signal at 520 nm.

recover the original Schiff-base character would dissipate the energy to the local environment. These two species may be responsible for DNA cleavage. We believed that the photocleavage may occur at the nucleobases in DNA and result in the cleavage of phophodiester bonds.[20]

Transient absorption experiments were carried out in an attempt to probe the proposed intermediate, i.e., a V-bound hydroxyl species **B**. Figure 5b shows the temporal evolution of transient absorption spectra for complex **3d** in air-saturated aqueous DMSO (50%). Obviously, a distinct absorption band maximized at ∼520 nm was observed, of which the relaxation dynamics could be well fitted by a single-exponential decay, and a lifetime of 1.54 μs was resolved (see the inset in Figure 5b). In marked contrast, no such band was observed by examining the corresponding vanadyl(IV) species under the same conditions. The transient absorption profile for **3d**, as well as the associated relaxation dynamics, remains unchanged after degassing. The O_2-independent spectral properties eliminate the possibility that the 520 nm transient absorbance results from the triplet−triplet absorption. Alternatively, it is more plausible that 520 nm transient species be ascribed to the *o*-quinone methide intermediate **B** proposed in Figure 5a. Further in-depth investigations are underway to unravel the photocatalytic profiles of a series of the chiral oxometallic family based on these templates.

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Supporting Information Available: Preparations of complexes **1**−**7**, gel electrophoresis results, and X-ray crystallographic data for **2a** and **3b**−**3d** (CIF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(18) Direct assembly of complex **3d** by mixing the chiral Schiff base and sodium metavanadate or orthovanadate is feasible. However, the absolute configuration around the vanadium center cannot be controlled.

(19) (a) Fischer, M.; Wan, P. *J. Am. Chem. Soc*. **1999**, *121*, 4555. (b) Brousmiche, D. W.; Xu, M.; Lukeman, M.; Wan, P. *J. Am. Chem Soc*. **2003**, *125*, 12961.

(20) Piperidine treatment shows the enhancement in DNA cleavage. On the basis of the analysis of high-resolution polyacrylamide gel electrophoresis, DNA fragments induced by photocleavage did not show a doublet for each nucleotide, nor did they comigrate with $Fe(EDTA)^{2-}/H_2O_2$ cleavage products, thus excluding H atom abstraction from the C-4′ of the ribose core. See Figure S10 in Supporting Information for details.