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

Chien-Tien Chen,*† Jin-Sheng Lin,† Jen-Huang Kuo,† Shiue-Shien Weng,† Ting-Shen Cuo,† Yi-Wen Lin,† Chien-Chung Cheng,*,‡ Yan-Chen Huang,‡ Jen-Kan Yu,§ and Pi-Tai Chou*§

Department of Chemistry, National Taiwan Normal University, Department of Chemistry, Tamkang University, and Department of Chemistry, National Taiwan University, Taipei, Taiwan

chefv043@scc.ntnu.edu.tw

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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 photoinitiation² or in the presence of cofactors.³ When combined with H₂O₂, a number of transition metal complexes⁴ can facilitate oxidative⁵ or phosphate diester⁶ cleavage of DNA/RNA by

incipient (or metal-bound) hydroxyl radicals or peroxo species. Photocleavage of DNA by vanadium(V) bisperoxo complexes (vanadates) is noteworthy in view of the single oxygen-generation mechanism involved. 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)3 and oxometallic9 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 (>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 H2O2. 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, 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 (R1 = R2 = 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-salicylidene 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 ~ i-Bu-3c; compare lanes 2, 4, and 5 in Figure 2) in the test photocleavage. In contrast, serine-based (R = CH3OH, lane 6) system 3h is less reactive than threonine (R = CHCH2OH, lane 7) system 3i. On the other hand, among four different aromatic pendant groups examined, the noncoordinating phenylalanine-based vanadyl(V) complex 3d (R = PhCH2) was the most efficient mediator (compare lanes 8, 9, 11, and 12 in Figure 2).11b

(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.
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 (supercoupled) 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, 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 H2O2, sodium azide (a singlet oxygen quencher), or sodium benzoate (NaBz, a hydroxyl radical quencher). Remarkably, the cleavage process continued to operate anaerobically (lane 8, Figure 2). In the presence of 10-fold excess of NaN3 (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 H2O2 (50 mM) and 3d (5 mM) in a 10:1 ratio (lane 2, Figure 3). 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 H2O2 as the co-oxidant. Under this circumstance, singlet oxygen may be generated in situ and responsible for the DNA cleavage.

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. Preferential cleavages at the guanine residues of the HIV-27 were observed with higher loading (≥100 μM) of 3d. 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 H2O2. In addition, random photocleavage was observed with 3d in the presence of ≥290-fold excess of H2O2 (≥29 mM), presumably due to the generation of free O2. 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 H2O2, despite the fact that both the bulge and hairpin loop sites contain the same 5′-TC/CT-3′ sequence. In addition, the cleavage profile remains the same down to 0.1 μM for 3d. 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. 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. Complex 3d recrystallized from MeOH exhibits a distorted octahedral geometry with two extra ligands from MeOH.
(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 π−π stacking effect between the naphthalene template in 3d and the aromatic floor provided by the G9–C22 pair and the additional π−π 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 (λem = 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 H2O, results in the blue emitting (λem = 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 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 phosphodiester 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 O2-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.


(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−/H2O2 cleavage products, thus excluding H atom abstraction from the C-4′ of the ribose core. See Figure S10 in Supporting Information for details.

Figure 4. (a) Chem-3D presentation for the X-ray structure of 3a−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% polyacrylamide gel (7 M urea). Lane 1: DNA control with photocleavage; lane 2: Maxam–Gilbert G marker; lane 3, DNA and 3d with photocleavage; lane 4, DNA and 3d without photocleavage.

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.