Vanadium(V)-Peroxo Complexes:
A Study of Their Specific DNA-Photocleavage Activities and NMR Spectral Properties

SHEK Lai Kuen

A thesis submitted in partial fulfillment of the requirements for the degree of
Master of Philosophy

April 2001
Hong Kong Baptist University
Abstract

Vanadium(V)-peroxo complexes have recently been shown to cleave DNA oxidatively via the production of singlet oxygen during their photolyses. In this study, nine diperoxo- complexes of the formula NH₄[VO(O₂)₂(L-L)], where L-L = 2,2'-bipyridine, 1,10-phenanthroline, 5-amino-1,10-phenanthroline, 5-nitro-1,10-phenanthroline, 5-chloro-1,10-phenanthroline, 4-methyl-1,10-phenanthroline, 4,7-dimethyl-1,10-phenanthroline, 5,6-dimethyl-1,10-phenanthroline and 3,4,7,8-tetramethyl-1,10-phenanthroline, and two monoperoxo- complexes, [VO(O₂)(terpy)(H₂O)]ClO₄ and [VO(O₂)(4'-Cl-terpy)(H₂O)]ClO₄, where terpy = terpyridine and 4'-Cl-terpy = 4'-chloro-terpyridine, were synthesized and structurally characterized by ⁵¹V and 2-D (¹H,¹H)-COSY NMR spectroscopy. The X-ray crystal structure of [VO(O₂)(4'-Cl-terpy)(H₂O)]ClO₄ was also determined. For complexes with an asymmetric ancillary ligand such as 5-amino-1,10-phenanthroline, two isomeric forms, the axial (ax) and the equatorial (eq) isomers, with the pendant amino group pointing towards the axial and the equatorial direction, respectively, were identified when these complexes dissolved in aqueous media. The ratios of the two isomers in the equilibrium mixtures were also distinct for different complexes, e.g., for the [VO(O₂)₂(5-NH₂phen)]⁻ complex, the eq : ax ratio was 3 : 1 whereas for the [VO(O₂)₂(5-NO₂phen)]⁻ complex, the ratio was 1 : 1. Furthermore, in all these complexes, the chemical shifts of the protons adjacent to the pyridine nitrogen trans to the peroxo groups were found to be quite downfield, δ = 9.32-9.86 ppm, whereas the protons adjacent to the pyridine nitrogen trans to the oxo group ranged from 8.04-8.89 ppm only, indicating that the peroxo ligand is a more potent
electron-withdrawing group than the oxo ligand. Detailed assignments of the NMR spectra of these complexes were made.

The interactions between four representative vanadium(V)-peroxo complexes and mononucleotides, such as 5'-AMP, 5'-TMP, 5'-GMP, 5'-dGMP and 5'-dCMP, were also studied by 1H NMR spectroscopy in D2O under photo-irradiation. Distinctly new NMR features were found in the interactions between [VO(O2)2(5-NO2phen)]− and 5'-TMP as well as between [VO(O2)2(5,6-Me2phen)]− and 5'-dGMP and 5'-GMP, indicative of formation of new products. 5-Formyl-2'-deoxyuridine was identified as the major product of the photo-oxidation mediated by the [VO(O2)2(5-NO2phen)]− complex. For the 5'-dGMP and 5'-GMP, the 8-oxo-7,8-dihydro-2'-deoxyguanosine and 8-oxo-7,8-dihydroguanosine, was identified as products in their respective photo-reactions with the [VO(O2)2(5,6-Me2phen)]− complex using HPLC-electrochemical detection technique. Peak broadening was also observed in most of the interactions studied after a 5-15 min photo-irradiation period. The broadened peaks became sharpened again with > 15 min of photo-irradiation. This observation suggests the possible existence of a paramagnetic intermediate, perhaps V(IV) or V(III), which bound to the mononucleotide but became dissociated during its conversion to the final product.

Some vanadium(V)-peroxo complexes, such as [VO(O2)2(5,6-Me2phen)]−, have further been demonstrated to exhibit specific photo-modification towards a supercoiled plasmid DNA, pBluescript, at two distinct sites, 5'-ATC and 5'-TACC, in our previous study. Using a synthetic 33-mer oligodeoxyribonucleotide with a sequence identical to the original plasmid DNA segment containing these two
specific photo-modification sites in this study, we found a distinct photo-modification pattern in which the single-base G-sites on this single-stranded substrate were preferentially modified by all of the eight complexes studied. This pattern is characteristic of an attack by singlet oxygen, presumably produced from the photolysis of these complexes at neutral pH. More remarkably, single-base modification at the T-sites, comparable in magnitude to those observed on the G-sites, was also observed with one particular complex, the \([\text{VO}(\text{O}_2)_2(5\text{-NO}_2\text{phen})]^-\). This photo-modification of the thymine base, which is much more difficult to be oxidized than the guanine base, is quite uncharacteristic of any known singlet oxygen-DNA chemistry. No significant binding interaction between these complexes and the oligonucleotide was seen in the gel mobility shift assay conducted on both the single- and double-stranded substrate.

Supercoiling was shown to be a critical prerequisite in the observed specific photo-modification towards the 5'-ATC and 5'-TACC sites on the plasmid DNA. This notion was derived from the observation that when the supercoiled plasmid DNA was linearized by treatment with a restriction endonuclease \(A\mu I\), a photo-modification pattern reminiscent of the much less specific photo-modification pattern observed with the single-stranded 33-mer oligonucleotide was obtained. The highly specific photo-modification activity shown by the \([\text{VO}(\text{O}_2)_2(5,6\text{-Me}_2\text{phen})]^-\) complex towards the supercoiled pBluescript was interpreted in terms of a specific binding interaction between this complex and a supercoil-stabilized local secondary structure of the DNA.
# Table of Contents

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Declaration</td>
<td></td>
<td>i</td>
</tr>
<tr>
<td>Abstract</td>
<td></td>
<td>ii</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td></td>
<td>v</td>
</tr>
<tr>
<td>Tables of Contents</td>
<td></td>
<td>vi</td>
</tr>
<tr>
<td>List of Tables</td>
<td></td>
<td>x</td>
</tr>
<tr>
<td>List of Figures</td>
<td></td>
<td>xi</td>
</tr>
<tr>
<td>List of Abbreviations</td>
<td></td>
<td>xvi</td>
</tr>
</tbody>
</table>

**Chapter 1**  
**Introduction**  
1.1 Vanadium(V)-Peroxo Complexes: Their Structural and Physico-Chemical Properties  
1  
1.2 Biological Chemistry of Vanadium(V)-Peroxo Complexes  
7  
1.3 Photochemistry of Vanadium(V)-Peroxo Complexes in Acidic and Neutral Aqueous Media  
11  
1.4 DNA-Photocleavage Activities of Vanadium(V)-Peroxo Complexes at Neutral pH  
16  
1.5 Oxidative Strand Scission of Nucleic Acids  
18  
1.6 DNA Supercoiling and Supercoil-Stabilized Local Secondary Structures  
28  
1.7 Objectives and Approach of this Study  
33  

**Chapter 2**  
**NMR Study of Vanadium(V)-Peroxo Complexes and Their Interactions with Mononucleotides under Photo-Irradiation Conditions**  
2.1 Introduction  
35  
2.2 Materials  
37  
2.3 Experimental Details  
39
2.3.1 Instrumentation 39
2.3.2 Preparation of M[VO(O₂)₂(L-L)] 39
2.3.3 Preparation of [VO(O₂)(4'-Cl-terpy)(H₂O)]ClO₄ 39
2.3.4 Characterizations of Vanadium(V)-Peroxo Complexes 40
2.3.4.1 Infrared Spectroscopy 40
2.3.4.2 Determination of Peroxide Content in Complex 40
2.3.4.3 Analysis of Vanadium Content in Complex 41
2.3.4.4 Charge Estimation of the Complex 42
2.3.4.5 Determination of Molar Absorptivity of Complex in Aqueous Solution 42
2.3.5 ⁵¹V NMR Spectroscopy Study 43
2.3.6 ¹H NMR Spectroscopic Study 43
2.3.7 ¹H, ¹H-COSY NMR Study 44
2.3.8 X-Ray Crystallographic Analysis of the [VO(O₂)(4'-Cl-terpy)(H₂O)]ClO₄ Complex 45
2.3.9 ¹H NMR Study of the Interaction between Selected Vanadium(V)-Peroxo Complexes and Mononucleotides under Photo-Irradiation at 365 nm 46
2.4 Results and Discussion 47
2.4.1 Structural Characterizations of the Oxoperoxo(4'-chloro-2,2':6',2''-terpyridine)vanadium(V) Perchlorate 47
2.4.2 X-Ray Crystal Structure of the Oxoperoxo(4'-chloro-2,2':6',2''-terpyridine)vanadium(V) Perchlorate 49
2.4.3 NMR Study of Vanadium(V)-Peroxo Complexes 53
2.4.4 ¹H NMR Study of the Interaction between Selected Vanadium(V)-Peroxo Complexes and Mononucleotides under Photo-Irradiation at 365 nm 62
2.4.4.1 ¹H NMR Study of the Interaction between Selected Vanadium(V)-Peroxo Complexes and Thymidine 5'-Monophosphate under Photo-Irradiation at 365 nm 62

vii
Chapter 3 Specific DNA-Photocleavage Activities of Vanadium(V)-Peroxo Complexes

3.1 Materials 80

3.2 Experimental Details 81

3.2.1 Extraction and Purification of Plasmid DNA (pBluescript) 81

3.2.2 Preparation of the 10 mM Sodium Phosphate Buffer 82

3.2.3 Plasmid DNA-Relaxation Assay 82

3.2.4 Preparation of 5'-32P End-labeled 33-mer Synthetic Oligonucleotide 83

3.2.5 Preparation of the G-marker lane for the Maxam-Gilbert Sequencing Experiment 84

3.2.6 Analysis of DNA Modifications on the 33-mer Oligonucleotide by Maxam-Gilbert Sequencing Technique 85

3.2.7 Analysis of DNA Modifications on Supercoiled Plasmid DNA by Sanger Dideoxy Sequencing Technique 86

3.2.8 Gel Mobility Shift Assay of the 33-mer Synthetic Oligonucleotide 87

3.2.9 Preparation of the Linearized Plasmid DNA (pBluescript) by the Endonuclease Afl III 88
| 3.3 | Instrumentation | 89 |
| 3.4 | Results | 90 |
| 3.4.1 | Analysis of DNA Modification Pattern of the 33-mer Synthetic Oligonucleotide by Maxam-Gilbert Sequencing Technique | 90 |
| 3.4.2 | Gel Mobility Shift Analysis of the Photo-Modification of the 33-mer Synthetic Oligonucleotide by the Vanadium(V)-Peroxo Complexes | 100 |
| 3.4.3 | DNA-Photocleavage Activities of Vanadium(V)-Peroxo Complexes Towards the Linearized Plasmid DNA | 103 |
| 3.4.4 | Analysis of DNA Modifications Towards Supercoiled vs. Linearized Plasmid DNA by Sanger’s Dideoxy Sequencing Technique | 107 |
| 3.5 | Discussion | 112 |

Chapter 4  Conclusions  125

Chapter 5  References  128

Chapter 6  Appendices  142

Curriculum Vitae  164