MASTER'S THESIS

Vanadium(V)-peroxo complexes: a study of their specific DNA-photocleavage activities and NMR spectral properties

Shek, Lai Kuen

Date of Award:
2001

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 $NH_4[VO(O_2)_2(L\text{-}L)]$, 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, $[VO(O_2)(terpy)(H_2O)]ClO_4$ and $[VO(O_2)(4'\text{-}Cl\text{-}terpy)(H_2O)]ClO_4$, where terpy = terpyridine and 4'-Cl-terpy = 4'-chloro-terpyridine, were synthesized and structurally characterized by ^{51}V and 2-D (^1H,^1H)-COSY NMR spectroscopy. The X-ray crystal structure of $[VO(O_2)(4'\text{-}Cl\text{-}terpy)(H_2O)]ClO_4$ 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 $[VO(O_2)_2(5\text{-}NH_2phen)]^-$ complex,. the *eq* : *ax* ratio was 3 : 1 whereas for the $[VO(O_2)_2(5\text{-}NO_2phen)]^-$ 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 D_2O under photo-irradiation. Distinctly new NMR features were found in the interactions between $[VO(O_2)_2(5\text{-}NO_2phen)]^-$ and 5'-TMP as well as between $[VO(O_2)_2(5,6\text{-}Me_2phen)]^-$ 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 $[VO(O_2)_2(5\text{-}NO_2phen)]^-$ 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 $[VO(O_2)_2(5,6\text{-}Me_2phen)]^-$ 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 $[VO(O_2)_2(5,6\text{-}Me_2phen)]^-$, 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 $[VO(O_2)_2(5\text{-}NO_2phen)]^-$. This photo-modification of the thymine base, which is much more difficult to be oxidized than the guanine base, is quite *un*characteristic 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 *Afl III*, 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 $[VO(O_2)_2(5,6\text{-}Me_2phen)]^-$ 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