

MASTER'S THESIS

A study of the photo-oxidation of thymine and thymidine by the $\text{NH}_4[\text{VO}(\text{O}_2)_2(5\text{-NO}_2\text{phen})]$ complex

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**A Study of the Photo-Oxidation of Thymine and Thymidine
by the $\text{NH}_4[\text{VO}(\text{O}_2)_2(5\text{-NO}_2\text{phen})]$ Complex**

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Abstract

Recently, vanadium(V)-peroxo complexes have been shown by us to cleave DNA when photo-irradiated (365 nm) at neutral pH. Under these conditions, singlet oxygen, $^1\text{O}_2$, which can cause DNA strand breaks, was shown to be produced from the photolysis of these complexes. Using a 33-base oligonucleotide as the substrate, preferential photo-modification of the guanine (G) bases was observed in all the complexes studied by Maxam-Gilbert sequencing technique. This modification pattern is characteristic of the well-known $^1\text{O}_2$ -DNA chemistry. However, in one particular complex, the $\text{NH}_4[\text{VO}(\text{O}_2)_2(5\text{-NO}_2\text{phen})]$ complex (VPNO₂), comparable modification at the thymine (T) sites was also observed. This observed T-modification is remarkable because thymine, which has the highest redox potential (1.7 V *vs.* NHE at pH 7) among all nucleobases, could not be oxidized by $^1\text{O}_2$.

In this work, we attempted to elucidate the underlying mechanism of the photo-oxidation of thymine and thymidine mediated by the VPNO₂ complex by first characterizing the product profiles of these reactions. With thymine as the reaction substrate, three products, namely, 5-formyluracil (5-FoU, 82%), 5-hydroxymethyluracil (5-HmU, 16%) and 5-methylbarbituric acid (5-MBA, 2%) were separated, identified and quantified by HPLC, LC-ESI-MS and ^1H NMR techniques. Similar product profile was also found using thymidine as the substrate, i.e., 5-formyl-2'-deoxyuridine (5-FodU, major), 5-hydroxymethyl-2'-deoxyuridine (5-HmdU), thymidine glycol (dTG), 5-methylbarbituric acid (5-MBA), together with small amounts of free thymine base and possibly 2-deoxyribonolactone. These product profiles are *not* consistent with an $\bullet\text{OH}$ radical-mediated oxidation of thymine and thymidine where thymine glycol and thymidine glycol, respectively, are the major products (>65%) with 5-FoU and 5-HmU (<10% together) as the minor products.

Our observed product profiles, in fact, bear a closer resemblance to the product profile of the menadione (MQ)-mediated photo-oxidation of thymidine where a thymine radical cation was proposed as a primary intermediate. Transient absorption spectra obtained for the two systems, i.e., the MQ- and the VPNO₂-mediated reactions, showed a common transient intermediate that absorbed at 390 nm, which is characteristic of a thymine radical cation.

The observation of 5-FoU and 5-FodU as the major products of the thymine and thymidine photo-oxidation, respectively, is interpreted in terms of a facilitated deprotonation of the 5-CH₃ substituent of thymine due to the existence of an intermolecular CH- π interaction between the 5-NO₂-phenanthroline ligand of the complex and the 5-CH₃ hydrogen of thymine and thymidine. The small amount of free thymine base produced from thymidine was attributed to the C-1' deoxyribose oxidation caused by the •OH radical generated from the VPNO₂ complex upon photo-irradiation, which was demonstrated by EPR spin trapping technique. Using ⁵¹V NMR technique, the vanadium(V) complex was shown to be reduced in the course of this reaction.

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