DOCTORAL THESIS

A mechanistic study of the preferential photo-oxidation of the 5-CH3 substituent of thymine and thymidine mediated by NH4(VO(O2)2(5-NO2phen))

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A Mechanistic Study of the Preferential Photo-Oxidation of the 5-CH_3 Substituent of Thymine and Thymidine Mediated by $NH_4[VO(O_2)_2(5\text{-}NO_2phen)]$

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Abstract

The photo-oxidation product profiles of thymine (T) and thymidine (dT) mediated by the $NH_4[VO(O_2)_2(5\text{-}NO_2phen)]$ complex ($VPNO_2$, where 5-NO_2phen = 5-nitro-1,10-phenanthroline) were characterized and quantified using HPLC, GC-MS, ^{1}H (and ^{13}C) NMR, ESI-HRMS and compared to those obtained using a well-studied photo-oxidant, 2-methyl-1,4-naphthoquinone, also known as menadione (MQ). A preferential oxidation of the 5-CH_3 substituent over the commonly observed oxidation of the electron-rich C5–C6 double bond of thymine was observed in the $VPNO_2$-mediated reaction, affording, for example, 78% of 5-formyl-2'-deoxyuridine (5-FodU) *vs*. 12% of thymidine glycol from the photo-oxidation of dT. Thymine/thymidine radical cation, $[T\bullet]^+$, and thymine/thymidine allyl radical, derived from the 5-CH_3 deprotonation of $[T\bullet]^+$, were shown to be the key intermediates in this reaction by transient absorption spectroscopy, EPR-spin trapping technique, product profile comparison among thymine and its analogs, namely, 5-ethyluracil and 5-isopropyluracil, together with the substrate kinetic isotope effects (KIE). $^{18}O_2$- and $H_2{}^{18}O$-labeling experiments revealed a scrambling of the formyl oxygen in the 5-formyluracil (5-FoU) and 5-FodU products. This oxygen scrambling was shown to be due to a rapid hydration with the solvent water. The observed strong preference towards

5-CH_3 oxidation was interpreted to be the result of the formation of an association complex between T (and dT) and $VPNO_2$ (association constant K_a ~10 M^{-1}) based on (1) the upfield shifts observed in the 5-CH_3 and H6 protons of T and dT during NMR titrations with $VPNO_2$; (2) product quenching in the presence of 5-trifluoromethyluracil (5-CF_3U), an effective competitor for the π-stacking interactions between $VPNO_2$ and T (and dT); (3) effect of $VPNO_2$ concentration on product profile; and (4) DFT modeling calculation of the π-stacked adduct of T and $VPNO_2$, where one of the 5-CH_3 hydrogens of T was positioned within hydrogen bonding distance (d = 2.563 Å) to one of the NO_2-oxygens of $VPNO_2$, which presumably facilitated the 5-CH_3 deprotonation of the thymine/thymidine radical cation generated from a primary one-electron transfer step, leading to the preferential formation of the 5-CHO products observed.

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