J. Am. Chem. Soc. **1999**, *121*, 2927–2928

2927

Kinetics of 2′-Deoxyuridin-1′-yl Radical Reactions

Calvin J. Emanuel,[†] Martin Newcomb,*[,†] Carla Ferreri,[‡,§] and Chryssostomos Chatgilialoglu*[,§]

Department of Chemistry, Wayne State University
Detroit, Michigan 48202
Dipartimento di Chimica Organica e Biologica
Università di Napoli "Federico II"
Via Mezzocannone 16, I-80134 Napoli, Italy
I.Co.C.E.A., Consiglio Nazionale delle Ricerche
Via P. Gobetti 101, I-40129 Bologna, Italy

Received November 19, 1998

DNA C1′ radicals lead to abasic site damage with the formation of 2-deoxyribonolactone residues.[1] Such alkaline-labile lesions result in strand scission[1] and have been reported to be mutagenic and resistant to repair nucleases.[2] The mechanistic aspects of C1′ radical reactions under either anoxic or aerobic conditions are currently under dispute. Figure 1 shows the reaction manifold for C1′ radicals illustrated for the specific case of the 2′-deoxyuridin-1′-yl radical (**1**). Reaction of **1** with a thiol such as glutathione returns the initial nucleoside or its anomer, whereas reaction with oxygen gives a C1′ peroxyl radical (**3**) that can ultimately lead to 2-deoxyribonolactone (**6**). These reactions have been discussed over the past two decades,[3] but quantitative kinetic measurements were not possible. Synthetic advances led to nucleosides modified with photoreactive groups that are specific C1′ radical precursors, and Greenberg[4,5] and Chatgilialoglu[6] reported product studies from radical **1**, produced by photolysis of precursor **7**, that are consistent with the general pathway in Figure 1. ESR and UV spectra of radical **1** were recently reported, and computational results revealed structural details of this radical.[7] In this work, we report the application of laser flash photolysis (LFP) methods for measurements of the kinetics of reactions of radical **1** with thiols and of superoxide release from peroxyl radical **3**.

Radical **1** was produced by 266-nm laser photolysis of precursor **7** as previously described.[7] The initial cleavage process must produce the pivaloyl radical, $Me_3CC(O)\bullet$, and **1** as the major products because no further growth in the UV spectrum of **1** was observed with ns-resolution after initial production by the laser flash (Figure 2). The UV spectrum of radical **1** decays slowly in He-sparged solutions but faster in the presence of oxygen due to formation of peroxyl radical **3**. A rate constant (k_T) of 1×10^9 M^{-1} s^{-1} was reported for the reaction of **1** with O_2.[7]

When radical **1** was produced in He-sparged solutions containing thiols, the rates of signal decay increased due to formation of **2** (Figure 3A). Second-order rate constants for reactions of **1** at pH 7 and 20 °C were $(2.3 \pm 0.5) \times 10^6$ M^{-1} s^{-1} for 2-mer-

[†] Wayne State University.
[‡] Università di Napoli "Federico II".
[§] Consiglio Nazionale delle Ricerche.
(1) Pogozelski, W. K.; Tullius, T. D. *Chem. Rev.* **1998**, *98*, 1089–1107. Burrows, C. J.; Muller, J. G. *Chem. Rev.* **1998**, *98*, 1109–1151.
(2) For example, see: Povirk, L. F.; Goldberg, I. H. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 3182–3186. Povirk, L. F.; Houlgrave, C. W.; Han, Y.-H. *J. Biol. Chem.* **1988**, *263*, 19263–19266.
(3) Goldberg, I. H. *Acc. Chem. Res.* **1991**, *24*, 191–198. Pratviel, G.; Bernadou, J.; Meunier, B. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 746–769. von Sonntag, C.; Schuchmann, H.-P. In *Peroxyl Radicals*; Alfassi, Z. B., Ed.; Wiley: New York, 1997; pp 173–234.
(4) Goodman, B. K.; Greenberg, M. M. *J. Org. Chem.* **1996**, *61*, 2–3.
(5) (a) Tallman, K. A.; Tronche, C.; Yoo, D. J.; Greenberg, M. M. *J. Am. Chem. Soc.* **1998**, *120*, 4903–4909. (b) Tronche, C.; Goodman, B. K.; Greenberg, M. M. *Chem. Biol.* **1998**, *5*, 263–271.
(6) Chatgilialoglu, C.; Gimisis, T. *Chem. Commun.* **1998**, 1249–1250.
(7) Chatgilialoglu, C.; Gimisis, T.; Guerra, M.; Ferreri, C.; Emanuel, C. J.; Horner, J. H.; Newcomb, M.; Lucarini, M.; Pedulli, G. F. *Tetrahedron Lett.* **1998**, *39*, 3947–3950.
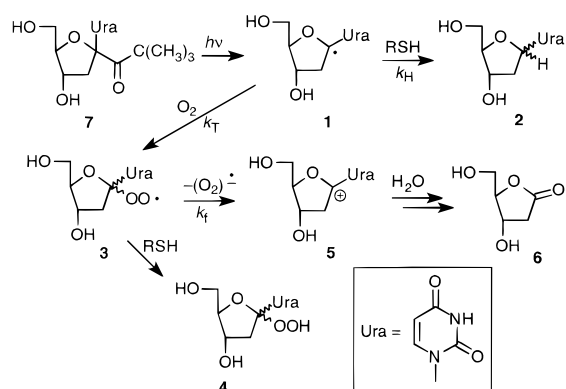

Figure 1. The 2′-deoxyuridin-1′-yl radical reaction manifold.
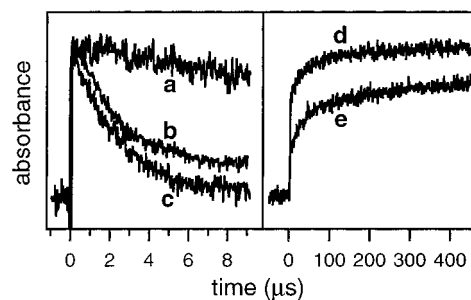

Figure 2. Left: Signal decay at 320 nm after photolysis of precursor **7** in (a) He-sparged water, (b) He-sparged water containing 0.1 M glutathione, and (c) nonsparged water ($[O_2] = 0.3$ mM). Right: Signal growth at 350 nm after photolysis of **7** (d) and di-*tert*-butyl ketone (e) in nonsparged methanol–water (1:99) containing 1.07×10^{-4} M TNM.
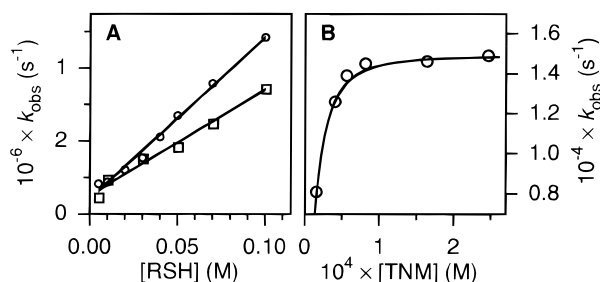

Figure 3. (A) Observed rate constants for decay of **1** in the presence of cysteine (squares) and glutathione (circles). (B) Observed pseudo-first-order rate constants for formation of nitroform anion in methanol–water (12:88, v:v) at 22 °C. The line is simulated pseudo-first-order behavior for release of superoxide radical anion with $k = 1.49 \times 10^4$ s^{-1} and reaction of $(O_2)^{\bullet-}$ with TNM with $k = 2.3 \times 10^9$ M^{-1} s^{-1}.

captoethanol, $(2.9 \pm 0.4) \times 10^6$ M^{-1} s^{-1} for cysteine, and $(4.4 \pm 0.3) \times 10^6$ M^{-1} s^{-1} for glutathione (errors at 2σ). The ratio of absolute rate constants for reaction of **1** with 2-mercaptoethanol and oxygen ($k_H/k_T = 2.3 \times 10^{-3}$) is in good agreement with the relative ratio found by Greenberg.[4]

Rate constants for heterolytic fragmentation of peroxyl radical **3** (k_f in Figure 1) to give superoxide radical anion, $(O_2)^{\bullet-}$, and cation **5** were determined from reactions conducted in the presence of tetranitromethane (TNM) which reacts with the superoxide radical anion to give the nitroform anion (eq 1) with λ_{max} at 350 nm.[8,9] The TNM detection method is complicated. High concen-

(8) Asmus, K.-D.; Henglein, A.; Ebert, M.; Keene, J. P. *Ber. Bunsen-Ges. Phys. Chem.* **1964**, *68*, 657–663. Hiller, K.-O.; Asmus, K.-D. *J. Phys. Chem.* **1983**, *87*, 3682–3628.
(9) Rabani, J.; Mulac, W. A.; Matheson, M. S. *J. Phys. Chem.* **1965**, *69*, 53–70.

trations of TNM are desired to avoid convolution of the pseudo-first-order rate constant for reaction of TNM with $(O_2)^{\bullet-}$ with the first-order rate constant for $(O_2)^{\bullet-}$ release, but TNM reacts rapidly with all reducing radicals[9,10] and competes with oxygen trapping reactions that give peroxyl radicals. An acceptable balance was found with TNM concentrations of about 1×10^{-4} M; convolution of the TNM reaction kinetics was minor (see below).

$$(O_2)^{\bullet-} + C(NO_2)_4 \rightarrow O_2 + (NO_2)\bullet + {}^-C(NO_2)_3 \quad (1)$$
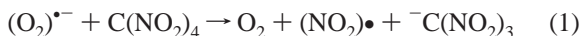
LFP studies were conducted with precursor **7** and with di-*tert*-butyl ketone (which gives pivaloyl and *tert*-butyl radicals upon photolysis) in the presence of O_2 and TNM, and signal growth at 350 nm was monitored. Nitroform anion was produced in two stages (Figure 2); fast signal growth observed in the first few microseconds from reactions of the initial radicals with TNM was followed by slower growth. For six independent studies with di-*tert*-butyl ketone in water at 25 °C,[11] the weighted average pseudo-first-order rate constant for nitroform anion formation in the slow process was $(0.95 \pm 0.04) \times 10^4$ s^{-1}. For six studies with precursor **7** under otherwise identical conditions, the weighted average pseudo-first-order rate constant was $(1.39 \pm 0.06) \times 10^4$ s^{-1}.

The slow nitroform-forming reaction observed with di-*tert*-butyl ketone is ascribed to release of either O_2 or $(O_2)^{\bullet-}$ from the pivaloylperoxyl radical, $(CH_3)_3CC(O)OO\bullet$, because fragmentation of the *tert*-butylperoxyl radical, $(CH_3)_3COO\bullet$, will be several orders of magnitude slower.[12] The same reaction(s) occurred in studies with precursor **7** because the pivaloylperoxyl radical was formed. The increased rate was due to reaction(s) of the C1′-peroxyl radical **3**. Loss of O_2 from **3** is likely to be slow (see below), and we ascribe the new reaction to superoxide release from **3**. The observed kinetics for nitroform anion formation with **7** are due to a mixture of reactions. From the amounts of signal growth in the fast and slow stages of nitroform anion formation (Figure 2), we conclude that oxygen trapping relative to initial TNM trapping is somewhat *less* efficient for the C1′ radical **1** than for the pivaloyl radical, and we estimate that the rate constant for heterolysis of **3** in water is approximately 2×10^4 s^{-1}.

Various LFP studies conducted with precursor **7** in water, and methanol−water solutions with varying concentrations of TNM supported the kinetic description. Nitroform anion production rates in the fast process were dependent on the concentration of TNM, and photolysis of **7** in He-sparged MeOH−H$_2$O (10:90, v:v) containing 1×10^{-4} M TNM at 24 °C gave a second-order rate constant for the reaction of all reducing radicals with TNM of 4×10^9 M^{-1} s^{-1}, similar to rate constants reported for reactions of α-heteroatom-substituted alkyl radicals with TNM.[9,10] The pseudo-first-order rate constant increased with increasing TNM concentration in the low concentration regime but not in the high concentration regime (Figure 3), a signature of convolution of kinetic processes, and the observed kinetic behavior was simulated by the model of consecutive reactions using a rate constant for reaction of $(O_2)^{\bullet-}$ with TNM that is approximately equal to the value reported in the literature $(2 \times 10^9$ M^{-1} s$^{-1})$.[8] The methodology was confirmed by studying an $(O_2)^{\bullet-}$ release reaction

for which the kinetics are known.[13] Control reactions with TNM-containing blank solutions indicated that direct photolysis of TNM did not complicate the analysis.

Whereas the rate constant for $(O_2)^{\bullet-}$ release from peroxyl radical **3** found here is similar to that obtained for the related peroxyl radical **9**,[12,13] it is 4 orders of magnitude *larger* than that reported by Greenberg and co-workers from measurements of ^{18}O-label incorporation in product ribonolactone **6**.[5a] The origin of this large difference is not apparent.

The possible reactions of DNA C1′ peroxyl radicals are trapping by thiol to give a hydroperoxide (such as **4**), superoxide radical anion release that produces a cation (**5**) and, ultimately, 2-deoxyribonolactone (**6**), and O_2 release that returns the C1′ radical.[14] The rate constant for reaction of the hydroperoxyl radical (HOO•) with thiol is about 120 M^{-1} s^{-1},[15a] and an upper limit for the rate constant for reaction of DNA-peroxyl radicals with glutathione was ≤ 400 M^{-1} s^{-1}.[15b] Therefore, at physiological concentrations of glutathione of about 5 mM, superoxide radical anion release from C1′ nucleotide peroxyl radicals is orders of magnitude faster than peroxyl trapping, and DNA C1′-hydroperoxides are not formed. Rate constants for loss of O_2 from allylperoxyl,[16a] cumylperoxyl,[16b] and nucleoside-C4′-peroxyl[16c] radicals are on the order of $1-2$ s^{-1}, or 4 orders of magnitude smaller than the rate constant for $(O_2)^{\bullet-}$ release from **3**. Because the stability of the C1′ radical is expected to be similar to that of a C4′ radical on the basis of poor delocalization of the unpaired electron into the base ring,[7] we believe that loss of O_2 from C1′-peroxyl radicals to give the C1′ radicals will not be competitive with $(O_2)^{\bullet-}$ release.

In summary, oxygen and glutathione trapping of C1′ radicals in nature are competitive processes due to the low O_2 concentration in the nucleus.[17] Once formed, the C1′ peroxyl radicals expel superoxide radical anion to give C1′ cations that lead to 2-deoxyribonolactone much faster than they can be trapped by glutathione to give hydroperoxides. The apparent difference in major reaction pathways for C1′ peroxyl radicals (superoxide release) and C4′ peroxyl radicals (release of molecular oxygen)[16c] is noteworthy.

(10) Eibenberger, J.; Schulte-Frohlinde, D.; Steenken, S. *J. Phys. Chem.* **1980**, *84*, 704−710. Schuchmann, H.-P.; von Sonntag, C. *Z. Naturforsch.* **1987**, *42B*, 495−502.

(11) Conditions: nonsparged 1% MeOH in water, $[O_2] \approx 3 \times 10^{-4}$ M, $[TNM] = 1.07 \times 10^{-4}$ M, 25 °C, data fit from 10 to 450 μs, errors at 2σ.

(12) Schuchmann, M. N.; Schuchmann, H.-P.; von Sonntag, C. *J. Am. Chem. Soc.* **1990**, *112*, 403−407. von Sonntag, C.; Schuchmann, H.-P. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1229−1253.

(13) Photolysis of radical precursor **8** in the presence of O_2 and TNM gave the pivaloylperoxyl radical and peroxyl radical **9**. The kinetic behavior in LFP studies with varying concentrations of TNM was similar to that in Figure 3B. For reactions in 30:70 MeOH−H$_2$O at 24 °C, the limiting value for $(O_2)^{\bullet-}$ release at high TNM concentrations was $2-3 \times 10^4$ s^{-1}, in good agreement with the value of 6×10^4 s^{-1} found in pulse radiolysis studies conducted in water.[12]
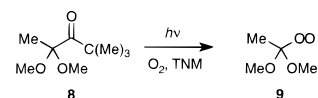
(14) Bimolecular reactions of two DNA C1′ peroxyl radicals[6] to produce 2′-deoxyribonolactone are unlikely due to the low probability that two macromolecular radicals can meet.

(15) (a) Lal, M.; Rao, R.; Fang, X.; Schuchmann, H.-P.; von Sonntag, C. *J. Am. Chem. Soc.* **1997**, *119*, 5735−5739. (b) Hildenbrand, K.; Schulte-Frohlinde, D. *Int. J. Radiat. Biol.* **1997**, *71*, 377−385.

(16) (a) Porter, N. A.; Mills, K. A.; Carter, R. L. *J. Am. Chem. Soc.* **1994**, *116*, 6690−6696. (b) Howard, J. A.; Bennett, J. E.; Brunton, G. *Can. J. Chem.* **1981**, *59*, 2253−2260. (c) Dussy, A.; Meggers, E.; Giese, B. *J. Am. Chem. Soc.* **1998**, *120*, 7399−7403.

(17) Zander, R. *Z. Naturforsch.* **1976**, *31C*, 339−352. Zander, R. *Adv. Exp. Med. Biol.* **1976**, *75*, 463−467.