

Oscillating Formation of 8-Oxoguanine during DNA Oxidation

Blánaid White,^{†,§} Malcolm R. Smyth,[†] James D. Stuart,[§] and James F. Rusling^{*,†,‡}

Department of Chemistry, University of Connecticut, U-60, 55 North Eagleville Road, Storrs, Connecticut 06269-3060, Department of Pharmacology, University of Connecticut Health Center, Farmington, Connecticut 06032, and National Centre for Sensor Research (NCSR), School of Chemical Sciences, Dublin City University, Dublin 9, Ireland

Received January 24, 2003; E-mail: james.rusling@uconn.edu

Genetic material undergoes oxidative stress that damages DNA bases.¹ Although nature has evolved diverse strategies to minimize its effects, such damage can interfere with normal replication.² Oxidative DNA damage occurs from irradiation, chemical reactions, and oxidation with reactive oxygen species (ROS),³ including hydroxyl radicals. Reaction of DNA with $\bullet\text{OH}$ yields 8-oxoguanine, 8-oxoadenine, thymine glycol, 8-hydroxycytosine, and strand breaks that may participate in mutagenesis.⁴ The goal of the present study was to measure the rate of 8-oxoguanine formation during DNA oxidation and to investigate its ultimate fate. We found that 8-oxoguanine was formed from oxidation of both DNA and free guanine in oscillating concentrations reflecting a complex reaction pathway, most likely involving further oxidation to 5-guanidino-hydantoin.

Guanine has the lowest oxidation potential of the DNA bases.⁵ Oxidation product 8-oxoguanine is considered a clinical biomarker for oxidative DNA damage.⁶ 8-Oxoguanine has a lower oxidation potential than guanine.⁷ Thus, when 8-oxoguanine was inserted into DNA sequences, it was the preferred oxidization site.⁸

We began by oxidizing free guanine using Fenton reagent to generate $\bullet\text{OH}$ radicals from Fe^{II} and hydrogen peroxide⁹ and analyzed the products by liquid chromatography with sequential UV and electrochemical detectors (LC–UV–EC).^{6,10} All reactions were done in triplicate in pH 5.5 buffer. The UV detector at 254 nm detected guanine, and the electrochemical detector detected 8-oxoguanine at +550 mV versus Ag/AgCl.

Oxidation of guanine gave a decrease in guanine concentration along with maxima in 8-oxoguanine concentrations at 4 and 15 min (Figure 1). The maximum concentration was $\sim 70 \mu\text{M}$, corresponding to 9% of the initial guanine. Qualitatively similar results were found at 0.8 mM and $8 \mu\text{M}$ guanine. Oxidation of 8-oxoguanine with $\bullet\text{OH}$ showed a rapid exponential decrease in concentration with 95% reactant consumed in 5 min.

Figure 2 shows the ratio $[\text{8-oxoguanine}]/\{[\text{8-oxoguanine}] + [\text{guanine}]\}$. After a small peak at 5 min, peaks in this ratio repeated with a frequency of 15 min. If 8-oxoguanine were the only product, the denominator would remain constant, and this ratio should increase, regardless of the oscillations. However, after about 20 min, the overall trend underlying the oscillations was a decrease with time.

Salmon testes (ST) ds-DNA was then reacted with Fenton's reagent. Aliquots from the DNA reaction were acid hydrolyzed under vacuum at 140 °C for 30 min to give free nucleobases.¹¹ Again the 8-oxoguanine concentration oscillated. Figure 3 shows $[\text{8-oxoguanine}]/\{[\text{8-oxoguanine}] + [\text{guanine}]\}$ over 90 min. There is an initial peak at 5 min and clear peaks at 20 and 65 min. An overall decreasing trend underlies the oscillations.

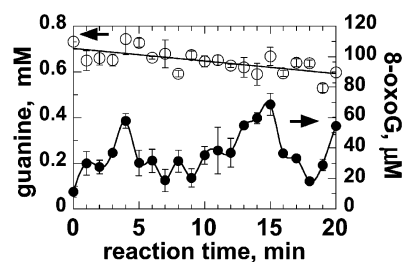


Figure 1. Concentration profiles of guanine (○) and 8-oxoguanine (●) from reaction of 0.8 mM guanine with $150 \mu\text{M}$ FeSO_4 and 50 mM H_2O_2 at 37 °C. (Lines in figures are cubic spline fits.)

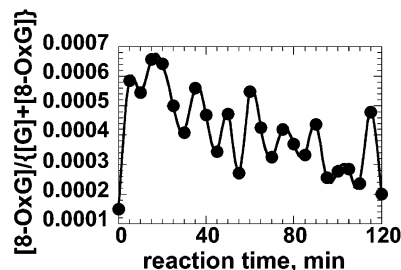


Figure 2. Ratio of $[\text{8-oxoguanine}]/\{[\text{8-oxoguanine}] + [\text{guanine}]\}$ for 0.8 mM guanine reacted with $150 \mu\text{M}$ FeSO_4 + 50 mM H_2O_2 , 37 °C.

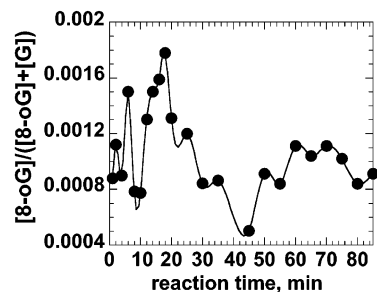


Figure 3. Ratio of $[\text{8-oxoguanine}]/\{[\text{8-oxoguanine}] + [\text{guanine}]\}$ for ST ds-DNA reacted with $150 \mu\text{M}$ FeSO_4 and 50 mM H_2O_2 , then hydrolyzed.

LC-MS was used to analyze ST ds-DNA incubated with Fenton reagent for 60 min, then hydrolyzed. LC-ion chromatograms showed peaks at m/z 152 $[\text{G} + \text{H}^+]$ at a retention time (t_R) of 2.9 min and m/z 158 $[\text{guanidino-hydantoin} + \text{H}^+]$ at t_R 3.1 min (see Supporting Information). The mass spectrum obtained from the LC eluant between 2.9 and 3.1 min showed peaks at m/z 152 and m/z 158, and at m/z 174 and m/z 190. The latter two peaks correspond to ions $[\text{G} + \text{Na}]^+$ (m/z 174) and $[\text{8-oxoG} + \text{Na}]^+$ (m/z 190), confirming the presence of 8-oxoguanine.

The results described above show that oxidation of guanine free or in ds-DNA by $\bullet\text{OH}$ leads to oscillating concentrations of 8-oxoguanine. The frequency for free guanine (one peak/15 min)

[†] University of Connecticut.

[‡] University of Connecticut Health Center.

[§] Dublin City University.

is larger than that for DNA (Figures 1–3), consistent with a smaller reaction rate for ds-DNA in which guanine is partly protected from oxidation within the double helix. Oscillations in the ratio $[8\text{-oxoguanine}]/\{[8\text{-oxoguanine}] + [\text{guanine}]\}$ were superimposed on a general decreasing trend at longer times, consistent with the oxidation of 8-oxoguanine. LC-MS detected 8-oxoguanine and guanidinohydantoin in oxidized DNA, indicating guanidinohydantoin as the oxidation product of 8-oxoguanine, as found previously.⁷

Our results are consistent with a competitive consecutive process in which guanine is oxidized to 8-oxoguanine, which is oxidized to guanidinohydantoin. Here, the common oxidant $\bullet\text{OH}$ reacts with starting reactant as well as the initial reaction product. This simple mechanism¹² is unlikely to lead to oscillating concentrations of initial product 8-oxoguanine. Oscillatory reactions typically have very complex pathways featuring interactive catalytic cycles. For example, the chlorite-iodide reaction has 13 elementary steps,¹³ and the reaction of NADH with O_2 catalyzed by peroxidases has 37 elementary steps.¹⁴ Studies of oligonucleotide oxidation suggest that there are likely to be several other intermediates and products involved.^{7,8,15} Extensive study may be needed to elucidate detailed mechanistic features.

In summary, 8-oxoguanine was formed in oscillating concentrations from the oxidation of free guanine and guanine in ds-DNA by $\bullet\text{OH}$. In living systems, DNA repair, oxidative inhibition, and other factors may be involved in controlling amounts of oxidized nucleobases. Nevertheless, oscillatory pathways may need to be considered in assessing the clinical significance of 8-oxoguanine biomarker assays.

Acknowledgment. This work was supported by the National Centre for Sensor Research (NCSR), Enterprise Ireland (MS), and U.S. PHS grant no. ES03154 (JR) from NIEHS and NIH. Contents are solely the responsibility of the authors. We thank M. Tarun and L. Zhou for assistance.

Supporting Information Available: Seven figures giving examples of LC chromatograms, raw data on DNA oxidation and controls, LC-

MS, and full experimental details (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) (a) Cross, C. E.; Halliwell, B.; Pryor, W. A.; Ames, B. N.; Saul, R. L.; McCord, J. M.; Harman, D. *Ann. Intern. Med.* **1987**, *107*, 526–545. (b) Beckman, K. B.; Ames, B. N. *J. Biol. Chem.* **1997**, *272*, 19633–19636.
- (2) (a) Lindahl, T. *Nature* **1993**, *362*, 709–715. (b) Friedberg, E. C.; Wagner, R.; Radman, M. *Science* **2002**, *296*, 1627–1630.
- (3) (a) Halliwell, B.; Gutteridge, J. M. C. *Biochem. J.* **1984**, *219*, 1–14. (b) Halliwell, B. *Mutat. Res.* **1999**, *443*, 37–52. (c) Cadet, J.; Delatour, T.; Douki, T.; Gasparutto, D.; Pouget, J.-P.; Ravanat, J.-L.; Sauvaigo, S. *Mutat. Res.* **1999**, *424*, 9–21.
- (4) (a) Pryor, W. A. *Free Rad. Biol. Med.* **1988**, *4*, 219–223. (b) Cheng, K. C.; Cahill, D. S.; Kasai, H.; Nishimura, S.; Loeb, L. A. *J. Biol. Chem.* **1992**, *267*, 166–172. (c) Olinski, R.; Gackowski, D.; Foksinski, M.; Rozalski, R.; Roszkowski, K.; Jaruga, P. *Free Rad. Biol. Med.* **2002**, *33*, 192–200. (d) Floyd, R. A. *Carcinogenesis* **1990**, *11*, 1447–1450.
- (5) Steenken, S.; Jovanovic, S. V. *J. Am. Chem. Soc.* **1997**, *119*, 617–618.
- (6) (a) Shigenaga, M. K.; Ames, B. N. *Free Rad. Biol. Med.* **1991**, *10*, 211–216. (b) Halliwell, B. *Free Rad. Biol. Med.* **2002**, *32*, 968–974. (c) Gedik, C. M.; Boyle, S. P.; Wood, S. G.; Vaughan, N. J.; Collins, A. R. *Carcinogenesis* **2002**, *23*, 1441–1446.
- (7) (a) Goyal, R. N.; Jain, N.; Garg, D. K.; *Bioelectrochem. Bioenerg.* **1997**, *43*, 105–114. (b) Duarte, V.; Muller, J. G.; Burrows, C. J. *Nucleic Acids Res.* **1999**, *27*, 496–502.
- (8) Hickerson, R. P.; Prat, F.; Foote, C. S.; Burrows, C. J. *J. Am. Chem. Soc.* **1999**, *121*, 9423–9428.
- (9) (a) Aruoma, O. I.; Halliwell, B.; Gajewski, E.; Dizdaroglu, M. *J. Biol. Chem.* **1989**, *264*, 20509–20512. (b) Walling, C. A. *Chem. Res.* **1975**, *8*, 125–131.
- (10) (a) Floyd, R. A.; Watson, J. J.; Wong, P. K.; Altmiller, D. H.; Rickard, R. C. *Free Rad. Res. Commun.* **1986**, *1*, 163–172. (b) Helbock, H. J.; Beckman, K. B.; Shigenaga, M. K.; Walter, P. B.; Woodall, A. A.; Yeo, H. C.; Ames, B. N. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 288–293.
- (11) Mbindyo, J.; Zhou, L.; Zhang, Z.; Stuart, J. D.; Rusling, J. F. *Anal. Chem.* **2000**, *72*, 2059–2065.
- (12) Zuman, P.; Patel, R. *Techniques in Organic Reaction Mechanisms*; Wiley: New York, 1984; pp 96–100.
- (13) Epstein, I. R.; Kustin, K. *J. Phys. Chem.* **1985**, *89*, 2275–2282.
- (14) Scheeline, A.; Olson, D. L.; Williksen, E. P.; Horras, G. A. *Chem. Rev.* **1997**, *97*, 739–756.
- (15) Luo, W.; Muller, J. G.; Rachlin, E. M.; Burrows, C. J. *Chem. Res. Toxicol.* **2001**, *14*, 927–938.

JA0343252