Incorporation of ^{18}O into Glycolic Acid Obtained from the Bleomycin-Mediated Degradation of DNA: Evidence for 4′-Radical Trapping by $^{18}O_2$

G. H. McGall, L. E. Rabow, and J. Stubbe*

Department of Biochemistry, College of Agricultural and Life Sciences, University of Wisconsin—Madison Madison, Wisconsin 53706

J. W. Kozarich

Department of Chemistry and Biochemistry University of Maryland, College Park, Maryland 20742

Received December 15, 1986

The in vitro degradation of DNA by bleomycin requires prior activation of the drug with either Fe(II) and O_2 or Fe(III) and H_2O_2.[1] Activated bleomycin can induce two types of DNA lesions. Predominating under anaerobic conditions are lesions resulting from the release of free nucleic acid bases and which cause strand scission upon subsequent treatment with hydroxide ion.[2] These alkali-labile sites have recently been the focus of structural investigations using model oligonucleotide substrates.[3,4] Activated bleomycin also causes strand breaks at neutral pH provided that additional O_2 is furnished beyond what is required for drug activation.[1a,2] This O_2-dependent strand scission involves cleavage of the deoxyribose C3′–C4′ bond, producing base propenals[5] as well as 5′-phosphate[6] and 3′-phosphoglycolate[5,7] termini (Scheme I). Although the exact mechanisms of these reactions are uncertain, there is evidence which suggests that both are preceded by an initial hydrogen atom abstraction from C4′ of the deoxyribose ring.[8] It is proposed that subsequent insertion of either OH or O_2 at this position would lead to intermediates whose decomposition would give rise to the observed products.[5,7-9] We have undertaken ^{18}O-labeling studies to determine the origin of the oxygen atoms incorporated at C3′ and C4′ in these products. These studies presently enable us to report that a single oxygen atom from the O_2 that is consumed subsequent to drug activation becomes incorporated into the carboxylate group of the oligonucleotide 3′-phosphoglycolate products which accompany the O_2-dependent strand scission.

In a series of experiments DNA was combined with bleomycin, activated by one of several methods (vide infra), in solutions saturated with either $^{16}O_2$ or $^{18}O_2$ at 4 °C. Analysis for ^{18}O incorporation involved the release of glycolic acid from the 3′-termini of the resulting oligonucleotides by digesting reaction mixtures with P_1 nuclease and alkaline phosphatase. The glycolic acid was isolated on a small column of DEAE Sephadex, eluted with 1% formic acid solution, neutralized immediately with NH_4OH, and then lyophilized. From reaction mixtures containing

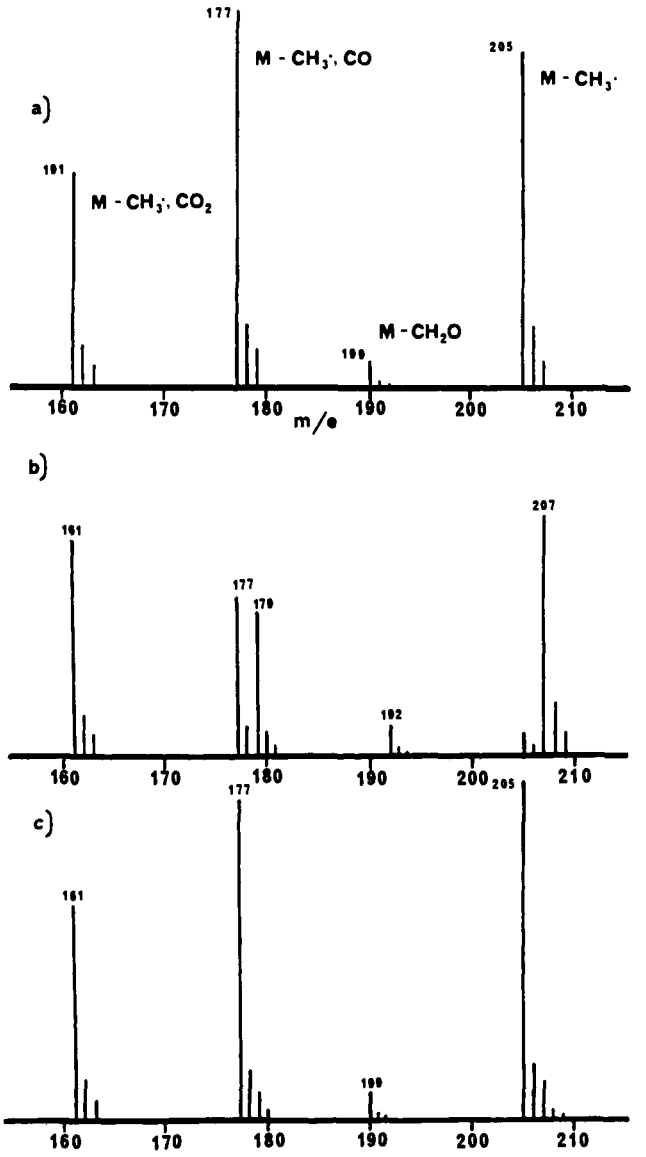
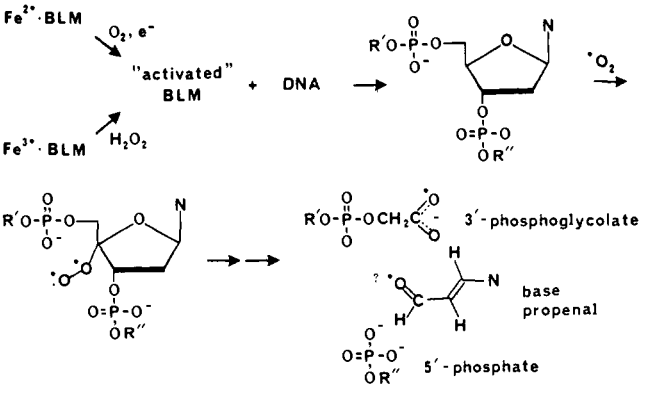


Figure 1. Partial mass spectra of the di-TMS derivative of glycolic acid obtained from the degradation of DNA with iron(II) bleomycin: (a) drug and DNA combined under $^{16}O_2$; (b) drug activated with $^{16}O_2$ and reacted with DNA under $^{18}O_2$; (c) drug activated with $^{18}O_2$ and reacted with DNA under $^{16}O_2$.

Scheme I. Degradation of DNA via a Bleomycin-Induced 4′-Radical[a]



[a] *O = ^{18}O.

500 nmol of DNA nucleotides, approximately 50 nmol of glycolic acid was recovered as the ammonium salt. As expected,[2b,5a,7b] this was equivalent to the total yield of base propenals as assayed by

* To whom correspondence should be addressed.

(1) (a) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1981**, *256*, 11636–11644. (b) Kuramochi, H.; Takahashi, K.; Takita, T.; Umezawa, H. *J. Antibiot. (Tokyo)* **1981**, *34*, 576–582. (c) Burger, R. M.; Blanchard, J. S.; Horwitz, S. B.; Peisach, J. *J. Biol. Chem.* **1985**, *260*, 15409.

(2) (a) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1982**, *257*, 3372–3375. (b) *Ibid.* **1982**, *257*, 8612–8614.

(3) (a) Wu, J. C.; Stubbe, J.; Kozarich, J. W. *Biochemistry* **1985**, *24*, 7569–7573. (b) Rabow, L.; Stubbe, J.; Kozarich, J. W.; Gerlt, J. *J. Am. Chem. Soc.* **1986**, *108*, 7130–7131.

(4) Sugiyama, H.; Xu, C.; Murugesan, N.; Hecht, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 4104–4105.

(5) (a) Giloni, L.; Takeshita, M.; Johnson, F.; Iden, C.; Grollman, A. P. *J. Biol. Chem.* **1981**, *256*, 8608–8615. (b) Burger, R. M.; Berkowitz, A. R.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1980**, *255*, 11832–11838.

(6) (a) Kross, J.; Henner, W. D.; Hecht, S. M.; Haseltine, W. A. *Biochemistry* **1982**, *21*, 4310–4318. (b) Kuo, M. T.; Haidle, C. W. *Biochem. Biophys. Acta* **1974**, *335*, 109–114.

(7) (a) Uesugi, S.; Shida, T.; Ikehara, M.; Kobayashi, Y.; Kyogoku, Y. *Nucleic Acid Res.* **1984**, *12*, 1581–1592. (b) Murugesan, N.; Xu, C.; Ehrenfeld, G. M.; Sugiyama, H.; Kilkuskie, R.; Rodriquez, L. O.; Chang, L.-H.; Hecht, S. M. *Biochemistry* **1985**, *24*, 5735–5744.

(8) (a) Wu, J. C.; Kozarich, J. W.; Stubbe, J. *J. Biol. Chem.* **1983**, *258*, 4694–4697. (b) Wu, J. C.; Kozarich, J. W.; Stubbe, J. *Biochemistry* **1985**, *24*, 7562–7568. (c) Ajmera, S.; Wu, J. C.; Worth, J.; Rabow, L.; Stubbe, J.; Kozarich, J. W. *Biochemistry* **1986**, *25*, 6586–6592.

(9) Grollman, A. P.; Takeshita, M.; Pillai, K. M. R.; Johnson, F. *Cancer Res.* **1985**, *45*, 1127–1131.

0002-7863/87/1509-2836$01.50/0

Table I. Incorporation of ^{18}O from $^{18}O_2$ into the Carboxyl Group of Glycolic Acid Obtained from the Products of Bleomycin-Mediated Cleavage of DNA

activating "pulse"	reaction "chase"	% (1-^{18}O)glycolic acid[a]
$^{18}O_2$/Fe(II)	$^{18}O_2$	98.1,[b] 92.4,[c] 95.5[d]
$^{18}O_2$/Fe(II)	$^{16}O_2$	3.5[b]
$^{16}O_2$/Fe(II)	$^{18}O_2$	89.7[b]
H_2O_2/Fe(III)	$^{18}O_2$	94.8[b]

[a] Percent enrichment relative to O_2 in the chase. [b] DNA from calf thymus. [c] poly d(AT). [d] d(CGCGCG).

the thiobarbituric acid method.[5] The glycolate salt was converted to a di-TMS derivative by heating at 60 °C with 10-20 μL of 50% *N,O*-bis(trimethylsilyl)trifluoroacetamide in acetonitrile. Aliquots of 1-5 μL were then analyzed directly by GC-MS. Relative amounts of unlabeled and (1-^{18}O)glycolic acid were determined from the average intensities of the M-CH_3 ions of the silylated derivatives at *m/e* 205 and 207, respectively, after correcting the mass spectrum for the natural abundance of the other stable isotopes.[10]

When iron(II) bleomycin[11,12] was allowed to react with calf thymus DNA, poly-d(AT), or d(CGCGCG) under an atmosphere of $^{18}O_2$, 92-98% of the isolated glycolic acid was singly labeled with ^{18}O at C1 (Figure 1). No label was incorporated at C2, as indicated by the lack of enrichment at *m/e* 161,[13] nor was any doubly labeled product observed. Control experiments using (1,1-$^{18}O_2$) glycolic acid showed that approximately 2-5% of the label is lost due to exchange with solvent during the workup. We therefore believe that these values reflect full incorporation of a single atom from O_2.

In order to distinguish between the O_2 involved in drug activation and the second O_2 required for the formation of 3′-phosphoglycolate termini, a "pulse-chase" method was employed. In a typical experiment,[12] an anaerobic solution of iron(II) bleomycin was activated with a "pulse" of $^{16}O_2$ or $^{18}O_2$.[14] After allowing 60 s for all of the iron(II) bleomycin to be consumed,[1a,b] the solution was evacuated briefly and purged with $^{18}O_2$ or $^{16}O_2$ and then an anaerobic solution of calf thymus DNA was immediately added. In another experiment, DNA was combined with iron(III) bleomycin and H_2O_2 under $^{18}O_2$.[16] Under these conditions, molecular oxygen does not participate in drug activation.[1a] The results of these experiments, summarized in Table I, clearly demonstrte that the oxygen incorporated at deoxyribose C-4′ is primarily, if not exclusively, derived from the second O_2 requirement and not the bound oxygen of activated bleomycin.[1a] This supports current hypotheses[8,9] which contend that C3′-C4′ bond cleavage is initiated by the addition of O_2 to a bleomycin-induced deoxyribose C4′ radical. The course of the reaction beyond this step remains somewhat obscure. It is frequently speculated that the resulting peroxyl radical is reduced to form a 4′-hydroperoxide which then undergoes a Criegee-type rearrangement[17] or some similar decomposition[8,9,18] resulting in C3′-C4′ bond cleavage. Further experiments intended to elaborate these points are in progress.

Acknowledgment. Supported by NIH grant GM-34454 (to J.W.K. and J.S.). J.W.K. is and American Cancer Society Faculty Research Awardee (1983-1988), and J. S. is an NIH Career Development Awardee (AM 01222) (1983-1988) and recipient of an H. I. Romnes Award, University of Wisconsin.

(10) Berry, J. A.; Osmond, C. B.; Lorimer, G. H. *Plant Physiol.* **1978**, *62*, 954-967.
(11) Bleomycin sulfate (Blenoxane) was a gift of Bristol Laboratories.
(12) Typical final concentrations in experiments with iron(II) bleomycin: Tris buffer, pH 7.5, 10 mM; bleomycin, 1 mM; $Fe(NH_4)_2(SO_4)_2$, 0.9 mM; DNA, 1 mM in nucleotides in a total volume of 500 μL.
(13) Peterson, G. *Tetrahedron* **1970**, *26*, 3413-3428.
(14) The stoichiometry of O_2 consumption in the absence of DNA has been shown to be 0.5 mol of O_2 per mol of iron(II) bleomycin,[15] resulting in production of a 1:1 mixture of iron(III) and activated bleomycin.[1b] In these experiments, oxygen was added as a saturated solution in H_2O.
(15) Horwitz, S. B.; Sausville, E. A.; Peisach, J. In *Bleomycin. Chemical, Biochemical, and Biological Aspects*; Hecht, S. M., Ed.; Springer-Verlag: New York, 1979; p 170-183.
(16) Final concentrations in experiments with iron(III) bleomycin: Tris buffer, pH 7.5, 10 mM; bleomycin, 0.25 mM; $Fe(NH_4)(SO_4)_2$, 0.25 mM; H_2O_2, 0.75 mM; DNA, 1 mM in a total volume of 500 μL.
(17) Saito, I.; Morii, T.; Matsura, T. *Nucleic Acid Res. Symp. Ser.* **1983**, *No. 12*, 95-98.
(18) Burger, R. M.; Projan, S. J.; Horwitz, S. B.; Peisach, J. *J. Biol. Chem.* **1986**, *261*, 15955-15959.

A Highly Selective Zeolite Catalyst for Hydrocarbon Oxidation. A Completely Inorganic Mimic of the Alkane ω-Hydroxylases

Norman Herron* and Chadwick A. Tolman

Central Research and Development Department
E. I. du Pont de Nemours and Company[†]
Experimental Station
Wilmington, Delaware 19898

Received October 9, 1986

Oxidation chemists have long envied natural oxidation enzymes which can achieve remarkable control over oxidation selectivities while using molecular oxygen and a reducing cofactor at room temperature. The natural monoxygenase enzymes, cytochrome P 450, display the ultimate in substrate selectivity[1] (choosing between substrates of different size or shape), while the ω-hydroxylases are even more remarkable in their ability to regioselectively hydroxylate the terminal methyl group of unactivated alkanes.[2] Selectivity is imposed in these natural systems by virtue of selective substrate binding or orientation relative to the active oxidant by the protein itself. We have sought to mimic the high selectivities of these natural systems by using zeolite catalysts, utilizing the similarities between cavities in a zeolite and those in the protein tertiary structure of oxidizing enzymes.[3] We have now designed a system containing Pd(0) and Fe(II) in a zeolite which, in an oxygen/hydrogen atmosphere, should generate hydrogen peroxide at the palladium sites[4] and then use that peroxide to do Fenton[5] or Udenfriend[6] type chemistry at the iron sites on any organic substrate which is concurrently present in the pore system. Since such chemistry can be constrained to occur in such a shape-selective environment, we anticipated considerable selectivity in the ensuing oxidation products. We now wish to report just such a result in the competitive oxidation of cyclohexane/*n*-octane mixtures, where dramatic substrate selectivity is evident combined with a regioselectivity of *n*-alkane oxidation comparable to the that of ω-hydroxylases.

Iron(II) ion exchanged zeolite 5A[7] (Si/Al ~ 1.2) is subsequently ion exchanged with palladium(II) tetramine chloride, to give a material containing ~1 wt % Fe and ~0.7 wt % Pd.

[†] Contribution No. 4237.
(1) White, R. E.; Coon, M. J. *Annu. Rev. Biochem.* **1980**, *49*, 315. Gunsalus, I. C.; Sligar, S. C. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1978**, *47*, 1.
(2) Hamberg, M.; Samuelsson, B.; Bjorkhem, I.; Danielsson, H. *Molecular Mechanisms of Oxygen Activation*; Hayaishi, O., Ed.; Academic: New York, 1974; p 29.
(3) Our previous work has demonstrated the parallels between zeolite chemistry and that of biological systems: Herron, N.; Tolman, C. A.; Stucky, G. D. *J. Chem. Soc., Chem. Commun.* **1986**, 1521. Herron, N. *Inorg. Chem.* **1986**, *25*, 4714.
(4) Hooper, G. W. Br. Patent 1056121, 1967 to ICI. Dyer, P. N.; Mosely, F. U.S. Patent 4128627, 1978 to Air Products. Kim, L.; Schoenthal, G. W. Ger. Patent 2615625, 1976 to Shell. Izumi, Y.; Miyazaki, H.; Kawahara, S. U.S. Patent 4009252; 1977 to Tokuyama Soda. Eguchi, M.; Morita, N. *Kogyo Kagaku Zasshi* **1968**, *71*, 783.
(5) Groves, J. T.; van der Puy, M. *J. Am. Chem. Soc.* **1974**, *96*, 5274.
(6) Udenfriend, S.; Clark, C. T.; Axelrod, J.; Brodie, B. B. *J. Biol. Chem.* **1954**, *208*, 731. Hamilton, G. A. *J. Am. Chem. Soc.* **1964**, *86*, 3391.

0002-7863/87/1509-2837$01.50/0