efficient transfer for $r^* < 6$ Å and adduct ion formation whenever electron transfer is slow. The abrupt change from C_3H_8 to C_2H_6 in our work is somewhat surprising, but no account has been taken of possible neutral → ion geometry changes or of chemical forces at short range. The newly observed H^- transfer reaction is inefficient for $r' = 9.1$ Å and highly efficient for $r' = 4.6$ Å, which seems a sensible range dependence for transfer of a heavy particle.

Different pairs of transition-metal ions M^{2+} and organic neutrals can be chosen to systematically vary the exothermicities of certain products and thus the crucial locations of corresponding curve crossings. This will permit considerable control of the product branching. Although M^{2+} species are highly energetic, our initial results indicate that their chemistry will be surprisingly rich and selective.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research and to the National Science Foundation for support under Grant CHE-8302856.

Identification of the Alkaline-Labile Product Accompanying Cytosine Release during Bleomycin-Mediated Degradation of d(CGCGCG)

L. Rabow and J. Stubbe*

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

J. W. Kozarich and J. A. Gerlt

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

Received June 18, 1986

Recent results from our laboratory[1] on the mechanism of DNA degradation by bleomycin (BLM)[2] have allowed us to propose a mechanism for free nucleic acid base release (Figure 1a). This hypothesis is supported by the mechanistic studies from a number of laboratories,[1,3] which indicate that Fe(II) and O_2 or Fe(III) and H_2O_2 can combine with BLM to form "activated BLM", which upon interaction with DNA produces two types of monomeric products, base propenal and free base. While base propenal production[3e] requires activated BLM and additional O_2 and occurs with concomitant DNA strand scission,[3d] the production of base has no additional O_2 requirement and yields strand scission only after treatment with alkali.[3c,i] Our hypothesis predicts that **1** (Figure 1a) is the precursor to this alkali-mediated DNA strand scission. We wish to report that, using the self-complementary hexamer d(CGCGCG) and either Fe(III)·H_2O_2·BLM (anaerobic) or Fe(II)·O_2·BLM, we have identified and quantitated, for the first time, the carbohydrate moiety remaining subsequent to free-base release.[4]

The strategy used to isolate **1** is outlined in Figure 1b. Sodium borohydride trapping of **1** subsequent to isolation by HPLC is an essential feature of the scheme, due to the chemical instability of **1**. A typical reaction mixture containing 0.21 mM Fe(III)·BLM, 0.6 mM H_2O_2, and 0.07 mM double stranded d(CGCGCG) in 10 mM HEPES (pH 7.5) was incubated 1.25 h at room temperature under anaerobic conditions. Analysis of the product distribution by reverse-phase HPLC is shown in Figure 2a. The number of moles of **1** isolated varied between $^1/_3$ to $^1/_2$ the number of moles of cytosine released (Figure 2a, peak A). The material with a retention time of 14 min (**1**) was typically isolated in 35–50% yield and immediately reduced with $NaBH_4$ (NaB^3H_4) at pH 7.6. The reduced reaction product was then chromatographed on both reverse-phase HPLC to give an 88% recovery of **2** (Figure 2b) and anion-exchange Mono Q FPLC to give a 72% yield of a single symmetrical peak.

The material in peak **2** was then subjected to enzymatic digestion with P_1 nuclease and alkaline phosphatase and the resulting products were analyzed by reverse-phase HPLC (Figure 2c). In addition to deoxyguanosine (peak D) (25.5 min) and deoxycytidine (peak C) (17.5 min), an unknown peak with a retention time of 20 min was observed, which contained 1 mol of phosphate per mol of deoxyguanosine and coeluted with the radioactivity. On

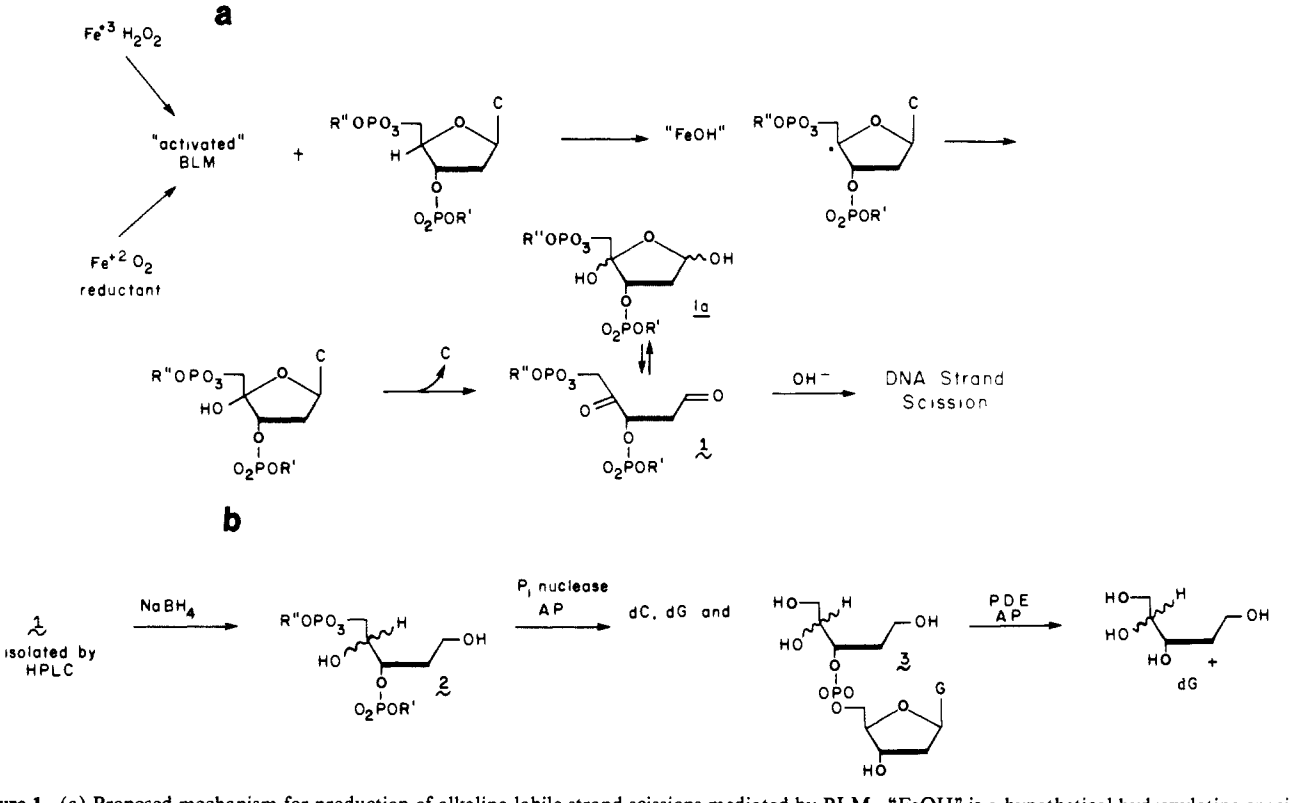

Figure 1. (a) Proposed mechanism for production of alkaline-labile strand scissions mediated by BLM. "FeOH" is a hypothetical hydroxylating species. Compound **1** could be in equilibrium with the ring closed hydrate **1a**.[3g] (b) Protocol for isolation of the carbohydrate moiety produced concomitant with cytosine release. AP, alkaline phosphatase; PDE, phosphodiesterase.

0002-7863/86/1508-7130$01.50/0

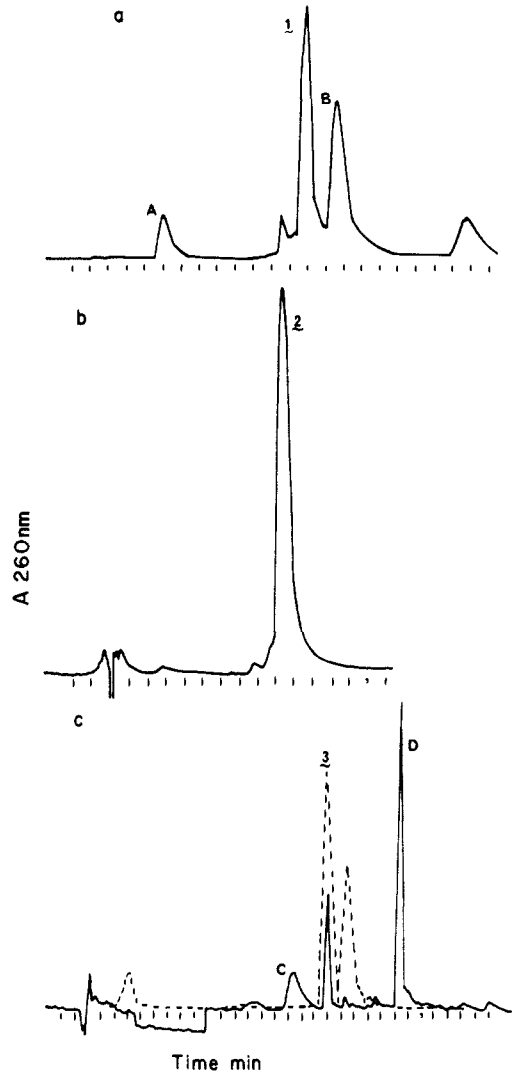

Figure 2. (a) HPLC analysis of Fe(III)·BLM·H_2O_2-treated d(CGCGCG). Separation was achieved on a C_{18} column using a linear gradient over 10 min from 0% to 20% CH_3OH in 5.0 mM potassium phosphate (pH 5.5); flow rate 1 mL/min. Compound, retention time, nmol: cytosine (A), 6 min, 33; "major peak" (1), 14 min, 12; d(CGCGCG) (B), 15.5 min, 9.4. (b) HPLC analysis of the product produced by $NaBH_4$ reduction of **1**, part a, to give **2**; Elution conditions, see above; retention time, 12 min. (c) The material in peak 2 (18 nmol) from Figure 2b was degraded with P_1 nuclease and alkaline phosphatase. Separation was achieved on a C_{18} column eluted isocratically for 5 min with 5.0 mM ammonium acetate (pH 5.5) followed by a 0–20% linear gradient in CH_3OH over 20 min. Compound, retention time, nmol: deoxycytidine (C), 17.5 min, 63.5; **3**, 20 min, 19; deoxyguanosine (D), 25.5 min, 64. (—) *A*, 260 nm; (---) ^3H as determined by scintillation counting.

the basis of the known specificity of P_1 nuclease,[5] compound **3** (Figure 1b) is the proposed structure. The material in peak **3** was shown to comigrate[6] with an authentic sample of one diastereomer of **3** prepared by degradation of d(CpG) by Maxam-Gilbert methodology.[7,8]

In addition, cleavage of [^3H]-**3** with snake venom phosphodiesterase resulted in the production of **4** and dGMP. The latter was identified by cochromatography with authentic dGMP by using an ion-pairing reverse-phase system. The carbohydrate moiety **4** eluted with a retention time of 3.5–4.5 min from a reverse-phase column with H_2O elution and was shown to comigrate in two solvent systems with the two diastereomers of **4** prepared by independent chemical syntheses.[9] The overall recovery of **3** from **1** was ~85%.

Similar experiments have also been completed with d(CGCGCG) and BLM, Fe(II), and O_2 to form activated BLM. The material corresponding to peak **1** has been isolated and identified along with other expected products from the O_2-dependent base propenal pathway.

These results indicate that activated BLM generated by either Fe(II) and O_2 or Fe(III) and H_2O_2 is capable of producing **1** (Figure 1) with concomitant free base release and are consistent with the hypothesis put forth by us[1] that free-base release is the result of 4′-hydrogen abstraction followed by 4′-hydroxylation.

Acknowledgment. Supported by NIH Grant GM-34454 (to J.W.K. and J.S.). J.W.K. is an 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.

* To whom correspondence should be addressed.

(1) (a) Wu. J. C.; Kozarich, J. W.; Stubbe, J. *Biochemistry* **1985**, *24*, 7562–7568. (b) Wu, J. C.; Stubbe, J.; Kozarich, J. W. *Biochemistry* **1985**, *24*, 7569–7573.

(2) Umezawa, H.; Maeda, K.; Takeuchi, T.; Okami, Y. *J. Antibiot. (Tokyo)* **1966**, *19A*, 200–209.

(3) (a) Sausville, E. A.; Peisach, J.; Horwitz, S. B. *Biochem. Biophys. Res. Commun.* **1976**, *73*, 814–822. (b) Burger, R. M.; Berkowitz, A. R.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1980**, *255*, 11832–11838. (c) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1981**, *256*, 11636–11644; (d) *Ibid.* **1982**, *257*, 8612–8614. (e) Giloni, L.; Takeshita, M.; Johnson, F.; Iden, C.; Grollman, A. P. *J. Biol. Chem.* **1981**, *256*, 8608–8615. (f) Rodriguez, L. O.; Hecht, S. M. *Biochem. Biophys. Res. Commun.* **1982**, *104*, 1470–1476. (g) Sugiyama, H.; Xu, C.; Murugesan, N.; Hecht, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 4104–4105. (h) Burger, R. M.; Blanchard, J. S.; Horwitz, S. B.; Peisach, J. *J. Biol. Chem.* **1985**, *260*, 15406–15409. (i) Burger, R. M.; Peisach, J.; Horwitz, S. B. *J. Biol. Chem.* **1982**, *257*, 3372–3375.

(4) Similar experiments have recently been reported by Sugiyama et al.[3g] In these experiments, alkali rather than $NaBH_4$ was used as a trap of **1** or **1a** (Figure 1) and no quantitation of products produced was reported.

(5) Fujimoto, M.; Kuninaka, A.; Yoshino, H. *Agric. Biol. Chem.* **1974**, *38*, 1555–1561.

(6) (a) C_{18}-reverse-phase (RP) chromatography: isocratic elution with 5 mM ammonium acetate (pH 5.5) for 5 min, followed by a linear gradient over 20 min to 20% CH_3OH; flow rate, 1 mL/min; retention time, 20 min. (b) Ion-pairing C_{18}-RP chromatography: isocratic elution with 88% 5 mM tetrabutyl ammonium bromide, 50 mM potassium phosphate (pH 4.8), and 12% CH_3OH; flow rate, 1 mL/min; retention time, 19.75–21 min. (c) C_{18}-RP chromatography: isocratic elution with H_2O; flow rate, 1 mL/min; retention time, 2.5 min.

(7) Cashmore, A. R.; Petersen, G. B. *Nucleic Acids Res.* **1978**, *5*, 2485–2491.

(8) DePuy, C. H.; Ponder, B. W. *J. Am. Chem. Soc.* **1959**, *81*, 4629–4631.

(9) 2-Deoxy-D-*erythro*-pentitol was prepared by $NaBH_4$ reduction of 2-deoxy-D-ribose. 2-Deoxy-L-*threo*-pentitol was prepared from methyl-β-D-xylopyranose via a five-step synthesis which will be reported elsewhere. ^1H NMR, ^{13}C NMR, and mass spectrometry (EI) of the Me_3Si derivatives are consistent with the proposed structures. Chromatographic separation (system, R_f): (a) cellulose plates impregnated with tungstate developed with acetone/1-butanol/H_2O (5:3:2), erythro isomer (0.58) and threo isomer (0.40); (b) silica gel plates impregnated with tungstate developed with ethyl acetate/isopropyl alcohol/H_2O (2:2:1), erythro isomer (0.41) and threo isomer (0.35).

Increase in the C=N Stretching Frequency upon Complexation of *trans*-Retinylidene-*n*-butylamine with General Lewis Acids

J. J. López-Garriga, G. T. Babcock,* and J. F. Harrison*

Department of Chemistry, Michigan State University East Lansing, Michigan 48824-1322

Received September 30, 1985

Part of the present knowledge on rhodopsin and bacteriorhodopsin photocycle intermediates comes from the resonance Raman behavior of the protein-bound retinal chromophore.[1] In vitro studies of retinal Schiff's bases and their protonated derivatives have been used effectively in showing that a protonated

0002-7863/86/1508-7131$01.50/0