Stereospecific 1,2-Hydride Shift in Ribonolactone Formation in the Photoreaction of 2′-Iododeoxyuridine

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The chemistry of DNA damage resulting from site-selective hydrogen abstraction from DNA deoxyribose by naturally occurring antitumor antibiotics[1] and designed synthetic DNA cleavers[2] has been a subject of much current interest. Understanding the detailed chemistry of site-specific and regiospecific H abstraction from the DNA sugar backbone provides extremely important information about the binding orientation of DNA-cleaving molecules in a duplex DNA.[3] Uracilyl-5-yl radical, a powerful H abstractor, can be readily generated at a desired site in a duplex DNA by incorporating 5-iodouracil (^{1}U) into the designed oligodeoxynucleotides followed by photoillumination.[4] Recently, we reported that photoirradiation of a ^{1}U-containing oligomer d(GCA^{1}UGC)$_2$ produces the deoxyribonolactone-containing hexamer **1** and erythrose-containing hexamer **2** with the release of free adenine and proposed a mechanism involving C1′ and C2′ H abstraction at the 5′ side of the ^{1}U residue of the same strand by the photochemically generated uracilyl-5-yl radical (Scheme 1).[4a] During our further investigation on the photoreaction of d(GCA^{1}UGC)$_2$, we observed that the ratio of **1** to **2** is highly dependent on the reaction conditions, particularly on the oxygen concentration. These results suggest the intriguing possibility that both **1** and **2** are produced via a common intermediate and led us to investigate the photoreactions of 2′α-iodo-2′-deoxyuridine ($^{2'}$IU) (**3**) and deoxyhexanucleotide d(GC$^{2'}$IUUGC) in an aqueous solution, since homolysis of the C−I bond of **3** would produce the C2′ carbon radical, which is essentially the same intermediate proposed to be formed in the photoirradiation of d(GCA^{1}UGC)$_2$.[4a]

A solution of **3**[5] in D_2O was photoirradiated in a Pyrex NMR tube (ϕ = 5 mm) with a transilluminator (302 nm) for 2 h under O_2-limiting conditions. The ^{1}H NMR spectrum of the reaction mixture revealed the presence of two pairs of double doublets (δ 2.59, 3.06 and δ 2.91, 3.23), suggesting the formation of deoxyribonolactone **4**[6] and 3′-ketodeoxyuridine **5**,[7] respectively (Figure 1a). Compound **5** was unstable in aqueous solution even at 0 °C and gradually decomposed to **6** and uracil with a $t_{1/2}$ of 48 h. Also, the ^{1}H NMR spectrum revealed the presence of characteristic signals (δ 4.06, 5.28, 6.20, and 7.68) ascribable to furanone **7**.[9] In addition to these products, the HPLC analysis of the reaction mixture revealed the production of other products, including uracil, **8**,[4a,10] **9**,[11] **10**, **11**, and **12**[12] (Scheme 2). The structures of **4**−**12** were confirmed by comparison of their HPLC profiles and spectral data with those of independently prepared authentic samples. These results clearly indicate that products **4** and **8**, which correspond to **1** and **2**, in the photoreaction of d(GCA^{1}UGC), respectively, are actually produced from the deoxyribose C2′ radical. Table 1 summarizes the product distribution for the photoreaction of **3** under several different sets of conditions.

The formation of **8** and major product **9** suggests the initial formation of the C2′ radical **13**, whereas the production of **10**, **11**, and **12** apparently indicates the intermediary formation of the C2′ carbocation **14**. The photoinduced homolytic cleavage of the C−I bond followed by rapid single electron transfer within a radical pair giving rise to ion pair formation has been well

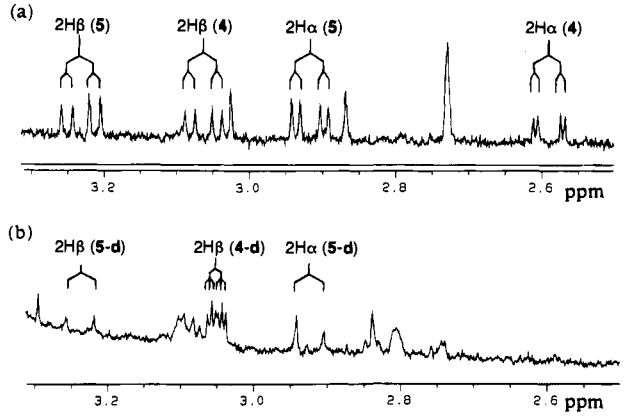
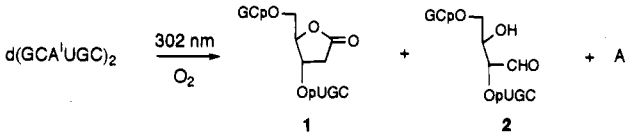
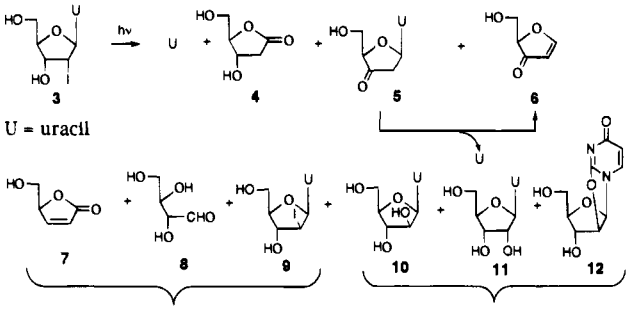


Figure 1. (a) ^{1}H NMR (400 MHz) spectrum of the photolysate of **3** in D_2O. (b) ^{1}H NMR spectrum of the photolysate of **3-d**. Each of the reaction mixtures (600 μL) containing **3** or **3-d** (10 mM) in a Pyrex NMR tube was irradiated at 0 °C with a transilluminator (302 nm) under O_2-limiting conditions for 2 h. The solution was degassed by flushing with argon.

Scheme 1



Scheme 2



(1) (a) Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107. (b) Goldberg, I. H. *Acc. Chem. Res.* **1991**, *24*, 191. (c) Hecht, S. M. *Acc. Chem. Res.* **1986**, *19*, 83.

(2) (a) Barton, J. K. *Science* **1986**, *233*, 727. (b) Strobel, S. A.; Dervan, P. B. *Science* **1990**, *249*, 73. (c) Sigman, D. S.; Chen, C.-H. B. *Annu. Rev. Biochem.* **1990**, *59*, 207.

(3) (a) Sugiyama, H.; Fujiwara, T.; Kawabata, H.; Yoda, N.; Hirayama, N.; Saito, I. *J. Am. Chem. Soc.* **1992**, *114*, 5573. (b) Sitlani, J.; Barton, J. K. *J. Am. Chem. Soc.* **1992**, *114*, 2303.

(4) (a) Sugiyama, H.; Tsutsumi, Y.; Fujimoto, K.; Saito, I. *J. Am. Chem. Soc.* **1993**, *115*, 4443. (b) Willis, M. C.; Hicke, B. J.; Uhlenbeck, O. C.; Cech, T. R.; Koch, T. H. *Science* **1993**, *265*, 1255.

(5) Abrams, D. N.; Knaus, E. E.; Wiebe, L. I.; Helus, F.; Maier-Borst, W. *Int. J. Appl. Radiat. Isot.* **1980**, *32*, 105.

(6) (a) Chem, S.-Y.; Joullie, M. M. *J. Org. Chem.* **1984**, *49*, 2168. (b) Urata, H.; Akagi, M. *Nucleic Acids Res.* **1991**, *19*, 1773.

(7) 3′-Keto-2′-deoxyuridine was prepared by the modified procedure of Hansske *et al.*[8] ^{1}H NMR (D_2O:CD_3COOD = 8:2): δ 2.88 (dd, 1 H, J = 19.4, 5.7 Hz, 2′a), 3.22 (dd, 1 H, J = 19.4, 7.9 Hz, 2′b), 3.90 (d, 1 H, J = 3.6 Hz, 5′), 4.35 (t, 1 H, J = 3.6 Hz, 4′), 5.90 (d, 1 H, J = 3.6 Hz, 5), 6.41 (dd, 1 H, J = 7.9, 5.7 Hz, 1′), 7.89 (d, 1 H, J = 7.7 Hz, 6).

(8) Hansske, F.; Madej, D.; Robins, M. J. *Tetrahedron* **1984**, *40*, 125.

(9) Camps, P.; Cardellach, J.; Font, J.; Ortuno, R. M.; Ponsati, O. *Tetrahedron* **1982**, *38*, 2395.

(10) Erythrose **8** was quantitated after derivatization to the (2,4-dinitrophenyl)hydrazone by means of HPLC.

(11) ^{1}H NMR (D_2O): δ 3.89 (dd, 1 H, J = 12.1, 4.5 Hz, 5′), 3.94 (ddd, 1 H, J = 7.5, 4.5, 2.5 Hz, 4′), 3.99 (dd, 1 H, J = 12.1, 2.5 Hz, 5′), 4.51(t, 1 H, J = 7.5 Hz, 3′), 4.75 (dd, 1 H, J = 7.5, 6.8 Hz, 2′), 5.94 (d, 1 H, J = 8.2 Hz, 5), 6.09 (d, 1 H, J = 6.8 Hz, 1′), 7.85 (d, 1 H, J = 8.2 Hz, 6). FABMS: $C_9H_{11}N_2O_5I$ *m/z* 355 (M + 1)$^+$.

(12) Hampton, A.; Nichol, A. W. *Biochemistry* **1966**, *5*, 2076.

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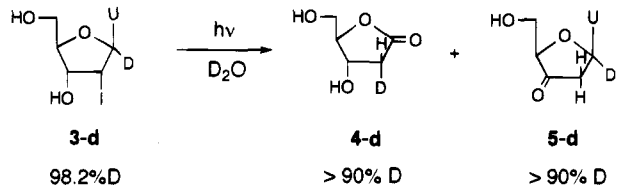
Table 1. Product Distribution in the Photoirradiation of **3**[a]

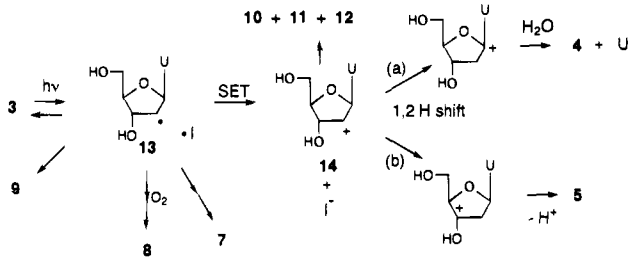
conditions	consumed **3** (%)	U (%)	C1′ oxid (%) **4**	C3′ oxid (%) **5**	**6**	C2′ radical product (%) **7**	**8**	**9**	C2′ carbocation product (%) **10**	**11**	**12**
aerobic, 4 h	37	7.0	1.0	1.0	0.5	2.0	0.8	13	3.0	1.0	2.0
O_2-limiting, 4 h	41	9.0	1.3	1.8	0.7	4.4	0.7	15	1.0	0.6	2.0
O_2-limiting, 8 h in CH_3CN[b]	60	11	1.5	4.0	*c*	*c*	2.0	10	*c*	*c*	*c*

[a] Each of the reaction mixtures (600 μL) containing **3** (10 mM) in a Pyrex NMR tube was irradiated as described in Figure 1. [b] **3** (10 mM) in acetonitrile. [c] Not detected.

Scheme 3



Scheme 4



established.[13] In the formation of **4**, no incorporation of deuterium from the D_2O solvent into the C2 position of **4** has been observed, implying that the C2 H is not derived from solvent water. In order to determine the origin of the C2 H of **4**, 1′-deuterated-2′α-iododeoxyuridine (**3-d**) (D content 98.2%)[14] was synthesized and subjected to photoirradiation under the same conditions. The ^1H NMR analysis of the photolysate indicated that the deuterium migrates to the C2α position of **4** (D content ~90%) (Figure 1b). The ^1H NMR analysis also showed the presence of **5-d** (D content ~90%) (Scheme 3) in the mixture. These results indicate that anomeric D of **3-d** migrated to the C2α position of **4-d**. Thus, the formation of **4** is explained by a stereospecific 1,2-shift of C1′ H to C2′α via carbocation **14** (Scheme 4, path a). Similarly, the 1,2-shift of C3′ H to the C2′ carbocation followed by deprotonation would produce **5** (path b).[15]

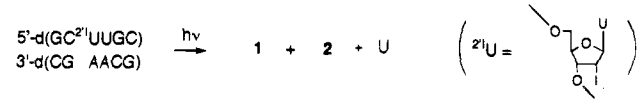
To determine whether such C1′ and C3′ oxidation products are actually formed via a C2′ radical intermediate like **13** in a duplex DNA, we have prepared deoxyhexanucleotide d(GC$^{2'}$IUUGC) (**15**) by the phosphoramidite method. Photoirradiation followed by HPLC analysis revealed that, in the presence of the complementary strand d(GCAAGC), **15** produced **1** (5.6%) and **2** (11%) as major products characterized under aerobic conditions (Scheme 5), whereas in the absence of d(GCAAGC), the photoirradiation of **15** gave numerous intractable products, implying that the double-stranded structure facilitates the formation of **1** and **2** from **15** (Table 2, runs 1 and 2). Under O_2-limiting conditions the yield of **1** increased with a slight decrease in **2** (run 3). This behavior is quite similar to that observed in the photoirradiation of d(GCA^{I}UGC)$_2$ (runs 4 and 5). While the contribution of the 1,2-shift during the formation of the 2-deoxyribonolactone residue in the photoirradiation of ^{1}U-containing DNA remains to be determined, the present results strongly suggest that a considerable portion of the deoxyribonolactone formation results from C2′ H abstraction.

Table 2. Formation of **1** and **2** in the Photoirradiation of Various Deoxyoligonucleotides[a]

run	oligomer	conditions	consumed hexamer (%)	U or A (μM)	**1** (%)	**2** (%)
1	**15**/d(GCAAGC)	aerobic	80[b]	65	5.6	11
2	**15**	aerobic	77	40	2.2	2.6
3	**15**/d(GCAAGC)	O_2-limiting	90[b]	37	7.6	9.5
4	d(GCA^{I}UGC)$_2$	aerobic	65	33[c]	10	9.7
5	d(GCA^{I}UGC)$_2$	O_2-limiting	70	29[c]	13	6.7

[a] Each of the reaction mixtures (50 μL) containing hexamer (1 mM) in 50 mM sodium cacodylate buffer (pH 7.0) was irradiated for 2 h. [b] Hexamer d(GCAAGC) was not consumed under the conditions. [c] Adenine was released.

Scheme 5



In summary, we have demonstrated for the first time that both C1′ and C3′ oxidation products are produced from the deoxyribose C2′ radical by a 1,2-hydride shift via the C2′ carbocation.

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JA9439231

(13) (a) McNeely, S.; Kropp, P. J. *J. Am. Chem. Soc.* **1976**, *98*, 4319. (b) Kropp, P. J.; Poindexter, G. S.; Pienta, N. J.; Hamilton, D. C. *J. Am. Chem. Soc.* **1976**, *98*, 8135.

(14) [1′-D]-2′α-Iododeoxyuridine (**3-d**) was prepared in nine steps from D-(+)-deoxyribonolactone.

(15) The mechanism of the formation of **7** is not clear at present. One possibility is heterolytic C−O bond cleavage of radical **13** at the C3′ position in a highly polar aqueous solvent followed by deprotonation and further oxidation, although previous model studies on the heterolytic β cleavage of the deoxyribose C4′ radical indicated that good anionic leaving groups like phosphate facilitate the heterolytic cleavage of the C−O bond in organic solvents.[16] In fact, **7** was not produced in the photoirradiation of **3** in dry acetonitrile (Table 1).

(16) (a) von Sonntag, C.; Hagen, U.; Schon-Bopp, A.; Schulte-Frohlinde, D. *Adv. Radiat. Biol.* **1981**, *9*, 109. (b) Giese, B.; Burger, J.; Kang, T. W.; Kesselheim, C.; Witter, T. *J. Am. Chem. Soc.* **1992**, *114*, 7322. (c) Giese, B.; Erdmann, P.; Giraud, L.; Gobel, T.; Petretta, M.; Schafer, T. *Tetrahedron Lett.* **1994**, *35*, 2683. (d) Giese, B.; Dussy, A.; Elie, C.; Erdmann, P.; Schwitter, U. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1861.