Pergamon

Tetrahedron Letters, Vol. 38, No. 46, pp. 8057-8060, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)10108-3

Preferential C1' Hydrogen Abstraction by a Uracilyl Radical in a DNA-RNA Hybrid

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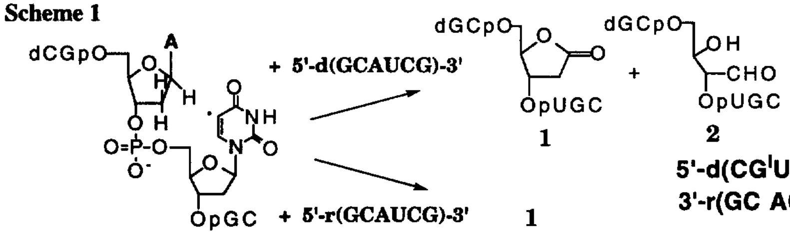
Abstract: Photoreaction of 5-halouracil contained in a DNA-RNA hybrid which forms an A form type structure was examined and the photoreactivity of this hybrid was compared with that for a DNA duplex. The results indicated that in a DNA-RNA hybrid, the uracilyl-5-yl radical preferentially abstracts the C1' H of the deoxyribose that is on the 5' side of the radical. NOE experiments of DNA hexamer and DNA-RNA hybrid demonstrated that there appears to be no correlation between the distances and the selectivity of H abstraction. In order to examine the origin of the selectivity of H abstraction, a putative transition state for H abstraction in a DNA duplex and a DNA-RNA hybrid was constructed and the conformational energy required to achieve their transition states was evaluated. The results provide a qualitative explanation of conformation-dependent H abstraction by the uracilyl-5-yl radical in a DNA duplex and DNA-RNA hybrid. © 1997 Elsevier Science Ltd.

The structure of DNA is well known to be remarkably heterogeneous.[1] DNA local conformations such as A form, Z form, and triplex, etc., have been suggested to play important biological roles such as gene expression through DNA-protein interactions.[2] Although several types of probes for DNA local conformation have been devised, there are only few techniques available for directly probing these local DNA structures in vivo.[1b,3] Because biologically important DNA local conformations are likely to appear in a very short period of time in certain cell cycles, a method that uses the DNA photoreaction to probe DNA conformation over a very short period of time would be infinitely useful. However, efforts to obtain DNA conformational information are impeded by the lack of knowledge of the detailed relationship between DNA conformation and photoreactivity. In a previous study, we found that photoirradiation of 5-halouracil-containing hexanucleotides yields C1'- and C2'-oxidation products on the 5' side of the 5-halouracil residue in B form DNA.[4] By using stereospecifically deuterated DNA oligomers, we have demonstrated that C2' oxidation of deoxyribose proceeds via a rate-limiting abstraction of 2'a-H and confirmed the existence of partitioning of C1' and C2' H abstraction reactions.[4d] These results imply that the uracilyl-5-yl radical competitively abstracts C1' and C2' H of the deoxyribose moiety on the 5' side of the 5-bromouracil residue,[5] suggesting that the reactivity of the uracilyl-5-yl radical generated in the fixed geometry of a DNA duplex should be highly dependent on the DNA conformation. The DNA-RNA hybrid which appears during transcription of DNA to mRNA and reverse transcription of RNA into DNA is known to form an A form type structure. Thus, in the present study, the photoreaction of 5-halouracil contained in a DNA-RNA hybrid was examined and the photoreactivity of this hybrid was compared with that for a DNA duplex. The results indicated that in a DNA-RNA hybrid, the uracilyl-5-yl radical preferentially abstracts the C1' H of the deoxyribose that is on the 5' side of the radical.

Photoirradiation of the DNA-RNA hybrid 5'-d(CGA^{Br}UGC)-3'/5'-r(GCAUCG)-3' which showed a typical A form CD spectrum was performed at 0 °C for 1 h using a transilluminator (302 nm). HPLC analysis of the photolysate indicated that C1' oxidation product **1** was obtained as a major product with release of free adenine. None of the C2' oxidation product, **2** was detected. Under the same conditions, photoirradiation of the DNA duplex 5'-d(CGA^{Br}UGC)-3'/5'-d(GCATCG)-3' yielded both C1' and C2' oxidation products as reported previously.[4] A similar result was obtained for a ^{I}U-containing DNA-RNA hybrid and DNA hexamer. Table 1 summarizes the product distribution in the photoirradiation of the hexanucleotides. The results clearly indicate that in the DNA-RNA hybrid the uracilyl-5-yl radical preferentially abstracts C1' H of the deoxyribose that is on the 5' side residue of the same strand as in Scheme 1.

Scheme 1



5'-d(CG^{I}UGT^{I}UTA^{I}UAC^{I}UG)-3'
3'-r(GC ACA AAT ATG AC)-5'
3

Table 1. Analysis of Products formed in Photoirradiation of 5-Bromouracil-Containing Deoxyhexanucleotides[a]

Run	Hexamer	A (μM)	Consumed hexamer (%)	C1' oxidation (%)	C2' oxidation (%)
1	d(CGA^{Br}UGC) r(GCU ACG)	28	41	34	0
2	d(CGA^{Br}UGC) d(GCT ACG)	25	38	20	6.8
3	d(CGA^{I}UGC) r(GCU ACG)	27	38	11	0
4	d(CGA^{I}UGC) d(GCT ACG)	27	33	6.7	7.4

[a] Each of the reaction mixtures (30 μL) containing hexamer (1 mM base concn) and NaCl (1 M) in 50 mM sodium cacodylate buffer (pH 7.0) in a capillary cell was irradiated for 3 h at 0 °C using a transilluminator (302 nm) under aerobic conditions.

A general feature of preferential C1' H abstraction in DNA-RNA hybrids was also confirmed by photoirradiation of ^{I}U-containing DNA-RNA hybrid 13 mer, **3**. Quantification of the C2' oxidation product at each reacted site was performed using a previously reported method.[4b] In a control experiment of photoirradiation of a DNA duplex 13 mer, the percentage of C2' oxidation varied from 10% to 77% depending upon the sites, whereas in a DNA-RNA hybrid 13 mer, C2' oxidation was significantly suppressed (>10%) at all four sites.

In agreement with a previous examination of DNA-RNA hybrid octamers by Reid *et al.*,[7] 2D NMR analysis of 5'-d(CGA^{Br}UGC)-3'/5'-r(GCAUCG)-3' performed in the present study indicated that the sugar conformations of a DNA strand have an O4' endo conformation.[8] NOE experiments of 5'-d(CGAUGC)-3'/5'-r(GCAUCG)-3' indicated that the distances between C5 of U4 and the C2' and C1' sugar protons of A3 are in the order of C2'α<C2'β<C1', whereas in 5'-d(CGAUGC)-3'/5'-d(GCATCG)-3' the order is C2'β<C2'α<C1'. Thus, there appears to be no correlation between the distances and the selectivity of H abstraction. In order to examine the origin of the selectivity of H abstraction, a putative transition state for H abstraction in a DNA duplex and a DNA-RNA hybrid was constructed and the conformational energy required to achieve their transition states was evaluated.[9] New sets of parameters of the transition state of H abstraction for the AMBER forcefield were prepared by ab initio molecular orbital calculation of the H abstraction of ethanol by vinyl radicals.[10] Next, decamers of the DNA duplex and DNA-RNA hybrid which contain putative H abstraction were minimized in the presence of 18 sodium cations and a 10Å layer of H_2O. The dinulceotide portion of the minimized structure that contains the transition states for C1', C2'α H abstraction in the DNA duplex, and C1' H abstraction in the DNA-RNA hybrid are shown in Figure 2.

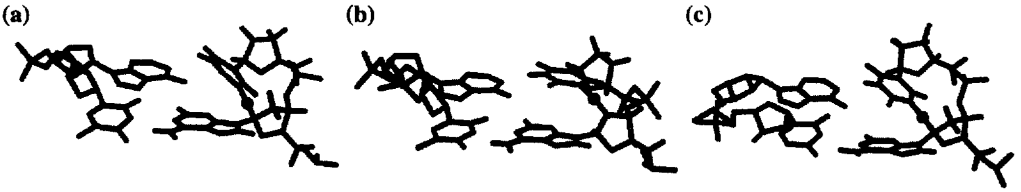


Figure 2. Structures of the A-U step for a minimized octamer which contains the putative transition state of C2'α (a) and C2'β (b) H abstraction in DNA duplex and C1' H abstraction in DNA-RNA hybrid (c).

Table 2 lists the minimized AMBER energies for putative transition structures for C1', C2'α and C2'β H abstraction in the DNA duplex and DNA-RNA hybrid. These results suggest that, in the DNA duplex, the conformational energy for C2'α H abstraction is the lowest AMBER energy among the other transition states.

Table 2. Minimized AMBER Total Energies (kcal/mol) of Decamer Containing Transition States for Hydrogen Abstraction by Uracilyl-5-yl Radical

	Transition state			
	C1'	C2'α	C2'β	Unmodified
5'-d(GCGCAUGCGC)-3' 3'-d(CGCGTACGCG)-5'	11.2 (17.5)[a]	6.5 (12.8)	27.7 (34.0)	-6.3
5'-d(GCGCAUGCGC)-3' 3'-r(CGCGUACGCG)-5'	18.7 (19.1)	32.8 (33.2)	26.4 (26.8)	-0.4

Starting structures were generated from standard B DNA and A DNA-RNA hybrid in a builder module of Insight II (MSI, San Diego, CA). The models were energy minimized by conjugate gradient (0.001 kcal/molÅ). Total energies of the molecules were evaluated using AMBER force field and distance-dependent dielectric of 4r after removing the counter ions and solvent. [a]The value in parentheses is the difference from the energy of the corresponding unmodified decamer.

Taking into account the intrinsic susceptibility of H abstraction due to a more stabilized C1' radical compared to that of C2' H abstraction, the activation energy level of C1' and C2'α transition states in B form are roughly equivalent, which would explain the observed competitive C1 and C2'α H abstraction.[12] The calculation also explains the selectivity for C2'α H abstraction over C2'β H abstraction in the DNA duplex. In the DNA-RNA hybrid, the minimized energy of the C1' transition state was the lowest, which explains the observed selective C1' H abstraction. While a more precise description of H abstraction would require dynamic aspects of nucleic acids to be accounted for, the present results provide a qualitative explanation of conformation-dependent H abstraction by the uracilyl-5-yl radical in a DNA duplex and DNA-RNA hybrid.

Acknowledgment. This work was supported by a Grant-in-Aid for Priority Research from the Ministry of Education, Science, Sports and Culture, Japan and the Research Foundation for Opto-Science and Technology.

References and Notes

1. (a) Cozzarelli, N. R.; Wang, J. C. Eds. *DNA Topology and Its Biological Effects,;* Cold Spring Harbor Laboratory Press: New York **1990.** (b) Sinden, R. R. Eds. *DNA Structure and Function;* Academic Press: NewYork **1994.**
2. Travers, A. A.; *Annu. Rev. Biochem.* **1989,** *58,* 427.
3. (a) Lilley, D. M. J. *Methods in Enzymology* **1992,** *212,* 133. (b) Sigman, D. S.; Chen, C. B.; *Annu. Rev. Biochem.* **1990,** *59,* 207. (c) Johnson, B. H., Rich, A. (1985) *Cell,* **42,** 713-724. (d) Barton, J. K. (1986) *Science,* **233,** 727-734. (e) Kirshenbaum, M. R., Tribolet, R., Barton, J. K. (1988) *Nucleic Acids Res.,* **16,** 7943-7960. (f) Mei, H.-Y., Barton, J. K. (1988) *Proc. Natl. Acad. Sci.,* **85,** 1339-1343.
4. (a) Sugiyama, H.; Tsutsumi, Y.; Saito, I. *J. Am. Chem. Soc.* **1990,** *112,* 6720. (b) Sugiyama. H.; Tsutsumi. Y.; Fujimoto. K.,; Saito. I. *J. Am. Chem. Soc.* **1993,** *115,* 4443. (c) Sugiyama. H.; Fujimoto. K.; Saito. I. *J. Am. Chem. Soc.* **1995,** *117,* 2945. (d) Sugiyama, H.; Fujimoto, K; Saito, I.; Kawashima, E.; Sekine, T.; Ishido, Y. *Tetrahedron Lett.* **1996,** *37,* 1805.
5. We[4c] and other groups[6] have recently indicated that in the photoreaction of $A^{Br}U$-containing oligomers a part of C1' oxidation product derived from C2' H abstraction via a 1,2-hydride shift of the C2' cation under anaerobic conditions. However, the results of deuterium labeling experiments have demonstrated that the major process by which the C1' oxidation product is obtained under aerobic condition derives from direct C1' H abstraction.
6. Cook, G. P.; Greenberg, M. M. *J. Am. Chem. Soc.* **1996,** *118,* 10025.
7. Salazar, M.; Fedoroff, O. Y.; Miller, J. M.; Ribeiro, N. S.; Reid, B. R. *Biochemistry,* **1993,** *32,* 4207.
8. Approximately the same magnitude of coupling constants between H1'-H2a (5.5 Hz) and H1'-H2b (5.7 Hz) and a strong NOESY cross peak between C1' and C4' indicating O4 endo conformation were observed.
9. Analogous incorporation of the transition state parameter into MM2 force field to evaluate the activation energy of various reactions has been reported. (a) Spellmeyer, D. C.; Houk, K. N. *J. Org. Chem.* **1987,** *52,* 959. (b) Wu, Y.-D.; Houk, K. N.; Florez, J.; Trost, B. M. *J. Org. Chem.* **1991,** *56,* 3656. (c) Damm, W.; Giese, B.; Hartung, J.; Hasskerl, T.; Houk, N. K.; Huter, O.; Zipse, H. *J. Am. Chem. Soc.* **1992,** *114,* 4067. (d) Brown, F. K.; Singh, U. C.; Kollman, P. A.; Raimondi, L.; Houk, K. N.; Bock, C. W. *J. Org. Chem.* **1992,** *57,* 4862.
10. Transition state analysis of the H abstraction of ethanol by a vinyl radical was peformed according to the reported procedure for H abstraction of ethanol by OH·.[11] Molecular orbital calculation was performed using Gaussian 92 and Spartan (version 3.1) programs on a Silicon Graphics IRIS Indigo R4400. The geometries of the transition state were obtained at UHF/6-31G* and the energies were estimated at the MP2/6-31G* level with consideration of zero point energy at the RHF/631G* level. Bond length of bifucated H (Hb)-sugar carbon (Cs) and Hb-uracil C5 (C5) were 1.38 Å and 1.32 Å, respectively, with corresponding H-C bond strength. Bond angle of C5-Hb-Cs is 180 ° with a bending force constant of 85 kcal which is the same as that for a normal C-C-C bond.
11. (a) Pardo, L.; Banfelder, J. R.; Osman, R. *J. Am. Chem. Soc.* **1992,** *114,* 2382. (b) Luo, N.; Kombo, D. C.; Osman, R. *J. Phys. Chem.* **1997,** *101,* 926.
12. The barrier for α and β H of ethanol was determined to be 8.5 and 11.7 kcal/mol, respectively. In agreement with a previous investigation, the present calculation suggests that C1' H abstraction is at least 3 kcal easier than that for C2' H abstraction.[13]
13. Miaskiewicz, K.; Osman, R. *J. Am. Chem. Soc.,* **1994,** *116,* 232.

(*Received in Japan* 8 *August* 1997; *revised* 11 *September* 1997; *accepted* 12 *September* 1997)