J|A|C|S
COMMUNICATIONS

Published on Web 05/06/2004

Facile Formation of an Intrastrand Cross-Link Lesion between Cytosine and Guanine upon Pyrex-Filtered UV Light Irradiation of d(^{Br}CG) and Duplex DNA Containing 5-Bromocytosine

Yu Zeng and Yinsheng Wang*

Department of Chemistry-027, University of California at Riverside, Riverside, California 92521-0403

Received January 14, 2004; E-mail: yinsheng.wang@ucr.edu

Partial replacement of thymidine with 5-bromo-2′-deoxyuridine in DNA increases considerably the sensitivity of living cells to UV light and γ irradiation.[1] It has been recognized that UV irradiation of 5-bromo-2′-deoxyuridine in DNA leads to the formation of strand breaks and alkali labile sites.[2−6] In this respect, detailed mechanism studies have been carried out and it has been shown that hydrogen abstraction from the deoxyribose of the adjacent 5′ nucleoside is important for the formation of the strand break.[3−6] In addition, 5-halo-2′-deoxyuridine has been used as a photoreactive probe for mapping the contact sites in protein−DNA complexes[7,8] and revealing the structures of nucleic acids.[9,10] The photochemistry of the relevant 5-bromo-2′-deoxycytidine, however, has not been extensively explored.[11−13] Herein we report for the first time the facile formation of an intrastrand cross-link lesion from Pyrex-filtered UV light irradiation of d(^{Br}CG) and duplex oligodeoxynucleotides (ODNs) containing 5-bromocytosine under aerobic condition.

Electrospray-ionization (ESI) mass spectrometry (MS) and tandem MS (MS/MS) show that the most abundant product isolated from the irradiation mixture of d(^{Br}CG) is an intrastrand cross-link lesion. Positive-ion ESI-MS of the HPLC fraction with a retention time of 15.9 min (LC trace shown in Figure 1) gives an ion of *m*/*z* 555.1, which is the $[M + H]^+$ ion of a product from d(^{Br}CG) with the elimination of an HBr moiety. MS/MS of the ion of *m*/*z* 555.1 shows that the most abundant fragment ion has a *m*/*z* of 261 (Figure S3a), which corresponds to the combined masses of cytosine and guanine after the loss of two hydrogen atoms. The presence of the *m*/*z* 261 ion demonstrates that the cytosine and guanine are covalently bonded. In addition, we observed a product ion resulting from the loss of a 2-deoxyribose (*m*/*z* 457). The types of fragment ions observed here have also been observed in the product-ion spectra of dinucleoside monophosphates containing intrastrand cross-link lesions involving adjacent 5-methylcytosine guanine[14] and two vicinal cytosines.[15]

^{1}H NMR spectrum of the cross-link lesion establishes unambiguously that the C5 carbon atom of cytosine and the C8 carbon atom of guanine are cross-linked and we designate the cross-link lesion as d(C[5-8]G). We observed a single aromatic proton at δ 8.21 ppm (Figure S4). This proton was attributed to the H6 proton of cytosine on the grounds that a strong correlation peak between this proton and the $H_{1'}$ proton of the 5′ nucleoside was found in the 2-D NOE spectrum (Figure S5). The absence of the H5 proton signal of cytosine and the H8 proton signal of guanine clearly demonstrates that the C5 carbon atom of cytosine and the C8 carbon atom of guanine are covalently bonded. We carried out the irradiation with four different time periods, 2, 5, 10, and 70 min, and, from the peak areas in the HPLC traces and the assumption that the cross-link lesion has a similar extinction coefficient at 260 nm as the starting material and other products, we estimated that the yields for the formation of d(C[5-8]G) are 1.9%, 6.0%, 13%, and 42%, respectively. The cross-link lesion can also form under anaerobic conditions, and the yields for its formation under aerobic and anaerobic irradiation conditions were similar (Figure S6). In analogy with our observation here, previous studies showed that photochemical activation of 5-bromo-2′-deoxycytidine gives rise to 5-aryl-2′-deoxycytidine and a 2′-deoxycytidine L-tryptophan cross-link.[12,13]

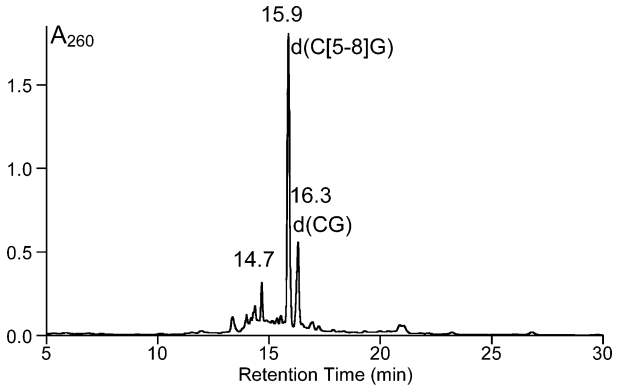


Figure 1. HPLC trace for the separation of the 70-min Pyrex-UV irradiation mixture of d(^{Br}CG) in the presence of air.

Interestingly, the same CG cross-link lesion in d(CGTA) has been isolated by Box and co-workers[16] from the X-ray irradiation mixture of the ODN under anaerobic conditions. These authors further proposed that the lesion was initiated from the dehydration of the coupling product of the 6-hydroxy-5-yl radical of cytosine and its adjacent guanine.[16] In this context, several intrastrand cross-link lesions that are initiated from a single pyrimidine radical have been reported.[14,16−22] The exact mechanism for the formation of the C[5-8]G cross-link lesion under Pyrex-filtered UV irradiation is not clear. An attractive mechanism (Scheme 1), however, can be proposed on the basis of previous studies of protein−nucleic acid cross-link induced by 5-bromo-2′-deoxyuridine.[23] Upon the Pyrex-filtered UV light irradiation, an electron can be transferred from guanine to 5-bromocytosine. The resulting anion radical of 5-bromocytosine may eliminate a bromide ion (Br^-) to give a C5 radical of cytosine, which can combine with the adjacent guanine cation radical. The coupling product can then deprotonate to yield the cross-link lesion (Scheme 1).

Next we examined whether a similar chemistry occurs in duplex DNA. To this end, we irradiated a 12-mer duplex, d(ATGGCG^{Br}-CGCTAT)/d(ATAGCGCGCCAT), under aerobic conditions, with the results showing that the most abundant product emanating from this irradiation is again a cross-link lesion (the HPLC trace for the separation of the 75-min irradiation mixture is shown in Figure 2).

We determined that the cross-link lesion has a covalent bond between the C8 carbon atom of the guanine at the sixth position and the C5 carbon atom of the cytosine at the seventh position on

10.1021/ja049760q CCC: $27.50

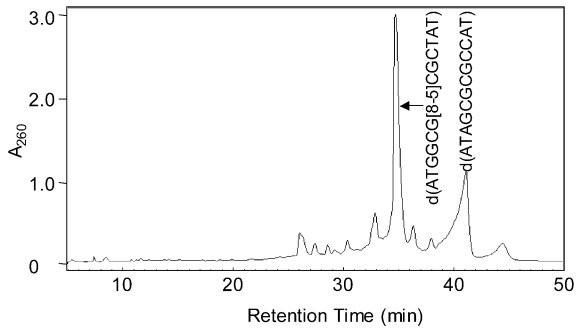
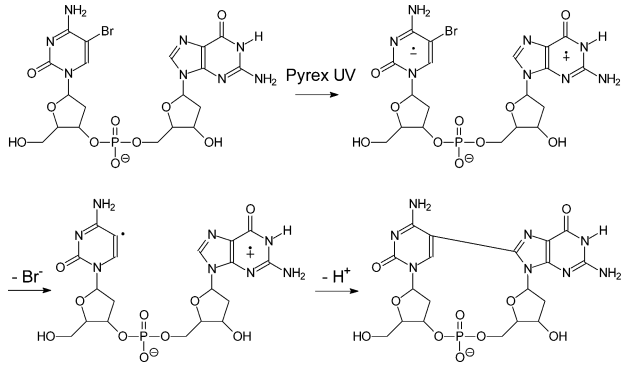


Figure 2. HPLC trace for the separation of Pyrex-filtered UV light irradiation of a duplex DNA under aerobic condition.

Scheme 1. Proposed Mechanism for the Formation of d(C[5-8]G)



the basis of the following lines of evidence. First of all, high-resolution ESI-MS acquired on a Fourier transform ion cyclotron resonance mass spectrometer gives *m*/*z* values of 1827.8077 and 1218.2093 for the $[M - 2H]^{2-}$ and $[M - 3H]^{3-}$ ions, respectively (the calculated *m*/*z* values for the $[M - 2H]^{2-}$ and $[M - 3H]^{3-}$ ions of the cross-link product are 1827.8077 and 1218.2055, respectively). Second, MS/MS of the $[M - 3H]^{3-}$ ion facilitates us to locate the site of the cross-link without ambiguity (Figure S9). Upon collisional activation in a mass spectrometer, an ODN undergoes cleavages at the glycosidic bond and the 3′ C−O bond of the same nucleotide to form $[a_n -$ base] and w_n ions.[24] The production of two complementary pairs of fragment ions, i.e., a_7^{2-}/w_5 and $[a_6 - G]$/$[w_6 + G]([w_6 + G]^{2-})$, from the cross-link-containing ODN demonstrates that the guanine at the sixth position and the cytosine at the seventh position are cross-linked because the formation of the a_7 and $[w_6 + G]$ ions can only occur when the two nucleobases are covalently bonded (Figure S9).

We further digested the cross-link lesion-containing dodecamer with nuclease P1 and alkaline phosphatase and isolated the resulting cross-link lesion-containing dinucleoside monophosphate by HPLC. ESI-MS/MS, ^{1}H NMR, and UV absorption spectroscopy show that the dinucleoside monophophate is d(G[8-5]C) (Figures S3b, S10, and S11).

It is worth mentioning that we were unable to isolate a cross-link lesion where the cytosine at the seventh position and the guanine at the eighth position are covalently bonded. The reason why this occurs is not very clear. Two factors, however, might contribute to this difference. Previous studies showed that, in B-DNA, the stacking energy is higher for 5′-purine−pyrimidine-3′ than for 5′-pyrimidine−purine-3′.[25] The more favorable stacking of 5-bromocytosine with its 5′ adjacent guanine than with its 3′ adjacent guanine is expected to lead to more facile electron transfer from 5′ guanine than from the 3′ guanine. In addition, the distance between the C5 carbon atom of cytosine and the C8 carbon atom of guanine in GC sequence context (3.87 Å) is shorter than that in CG sequence context (5.13 Å) in B-DNA (the distances were determined from a model duplex DNA that is created by using software package Insight II with standard B-DNA geometry, Figure S12). This may lead to more facile coupling between the C5 radical of cytosine and the cation radical of the adjacent guanine in the GC sequence context than in the CG sequence context (Scheme 1).

To our knowledge this is the first observation for the facile formation of intrastrand cross-link lesion from the photochemical activation of d(^{Br}CG) and 5-bromocytosine-containing duplex DNA. The chemistry uncovered here can be further used as a facile synthetic route for the construction of authentic CG and GC cross-link lesions for replication and repair studies.

Acknowledgment. We want to thank the National Institute of Health for supporting this research (R01 CA 96906).

Supporting Information Available: Experimental conditions and NMR and mass spectrometric characterizations. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Cadet, J.; Vigny, P. In *Bioorganic Photochemistry*; Morrison, H., Ed.; John Wiley: New York, 1990; Vol. 1, pp 1−272.
(2) Hutchinson, F. *Q. Rev. Biophys.* **1973**, *6*, 201−246.
(3) Sugiyama, H.; Tsutsumi, Y.; Saito, I. *J. Am. Chem. Soc.* **1990**, *112*, 6720−6721.
(4) Cook, G. P.; Greenberg, M. M. *J. Am. Chem. Soc.* **1996**, *118*, 10025−10030.
(5) Cook, G. P.; Chen, T.; Koppisch, A. T.; Greenberg, M. M. *Chem. Biol.* **1999**, *6*, 451−459.
(6) Chen, T.; Cook, G. P.; Koppisch, A. T.; Greenberg, M. M. *J. Am. Chem. Soc.* **2000**, *122*, 3861−3866.
(7) Willis, M. C.; Hicke, B. J.; Uhlenbeck, O. C.; Cech, T. R.; Koch, T. H. *Science* **1993**, *262*, 1255−1257.
(8) Hicke, B. J.; Willis, M. C.; Koch, T. H.; Cech, T. R. *Biochemistry* **1994**, *33*, 3364−3373.
(9) Sugiyama, H.; Fujimoto, K.; Saito, I. *Tetrahedron Lett.* **1997**, *38*, 8057−8060.
(10) Kawai, K.; Saito, I.; Sugiyama, H. *J. Am. Chem. Soc.* **1999**, *121*, 1391−1392.
(11) Prusoff, W. H. *Biochim. Biophys. Acta* **1962**, *58*, 588−590.
(12) Hassan, M. E. *Nucleosides Nucleotides* **1991**, *10*, 1277−1283.
(13) Celewicz, L. *J. Photochem. Photobiol., B: Biol.* **1995**, *30*, 129−137.
(14) Zhang, Q.; Wang, Y. *J. Am. Chem. Soc.* **2003**, *125*, 12795−12802.
(15) Liu, Z.; Gao, Y.; Wang, Y. *Nucleic Acids Res.* **2003**, *31*, 5413−5424.
(16) Box, H. C.; Budzinski, E. E.; Dawidzik, J. B.; Wallace, J. C.; Iijima, H. *Radiat. Res.* **1998**, *149*, 433−439.
(17) Box, H. C.; Budzinski, E. E.; Dawidzik, J. D.; Wallace, J. C.; Evans, M. S.; Gobey, J. S. *Radiat. Res.* **1996**, *145*, 641−643.
(18) Box, H. C.; Budzinski, E. E.; Dawidzik, J. B.; Gobey, J. S.; Freund, H. G. *Free Radical Biol. Med.* **1997**, *23*, 1021−1030.
(19) Budzinski, E. E.; Dawidzik, J. B.; Rajecki, M. J.; Wallace, J. C.; Schroder, E. A.; Box, H. C. *Int. J. Radiat. Biol.* **1997**, *71*, 327−336.
(20) Box, H. C.; Dawidzik, J. B.; Budzinski, E. E. *Free Radical Biol. Med.* **2001**, *31*, 856−868.
(21) Romieu, A.; Bellon, S.; Gasparutto, D.; Cadet, J. *Org. Lett.* **2000**, *2*, 1085−1088.
(22) Bellon, S.; Ravanat, J. L.; Gasparutto, D.; Cadet, J. *Chem. Res. Toxicol.* **2002**, *15*, 598−606.
(23) Meisenheimer, K. M.; Koch, T. H. *Crit. Rev. Biochem. Mol. Biol.* **1997**, *32*, 101−140.
(24) McLuckey, S. A.; Van Berkel, G. J.; Glish, G. L. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 60−70.
(25) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag New York Inc.: New York, 1984.

JA049760Q