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Formaldehyde-Mediated Modification of Natural Deoxyguanosine with Amines: One-Pot Cyclization as a Molecular Model for Genotoxicity

Hiroyasu Takahashi and Yuichi Hashimoto*

Institute of Molecular and Cellular Biosciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan

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Abstract—Stable cross-linked adducts, 3-(2-deoxy-β-D-ribofuranosyl)-7-phenyl-5,6,7,8-tetrahydro[1,3,5]triazino[1,2-*a*]purin-10(3*H*)-one and 7-butyl-3-(2-deoxy-β-D-ribofuranosyl)-5,6,7,8-tetrahydro[1,3,5]triazino[1,2-*a*]purin-10(3*H*)-one, that formed chemically from natural deoxyguanosine and aniline or buthyl amine in the presence of formaldehyde were identified. This reaction appears to be a general reaction of deoxyguanosine and primary amines, and it may be a model of DNA modification with carcinogenic aromatic amines without metabolic activation, if formaldehyde is present.

Formaldehyde is a widely used industrial chemical, and many people are exposed to it in new buildings, since the building materials release formaldehyde. The compound is now suspected to cause the so-called 'sick house' syndrome, which has become a major problem in Japan. In addition, various chemicals, including hexamethylphosphoramide (HMPA), dimethylaniline, cocaine, methylene chloride and so on, generate formaldehyde through cytochrome P450- or glutathione *S*-transferase-dependent metabolic pathways.[1,2] Formaldehyde is also well known as an embalming agent and as a genotoxic substance that causes DNA–protein or DNA–DNA cross-link formation.[3,4] Inhalation exposure to formaldehyde vapor induces carcinomas in rat nasal cavity,[5] and HMPA and methylene chloride are also mutagenic themselves.[2,6] Formaldehyde-induced DNA–protein cross-links have been suggested to induce mutation because of their ability to arrest DNA replication.[7] However, it was recently shown that formaldehyde-induced DNA–protein cross-links do not generate gene mutations in mammalian cells, though they seem to be related to cytotoxicity and clastogenicity.[8] This suggests that some other mechanism(s) may account for the mutagenic activity of formaldehyde.

Reaction of nucleic acids with formaldehyde, which is considered to be essential for genotoxicity of formaldehyde, has been studied extensively.[9] However, the chemical structures of the modified nucleic acids or adducts obtained when nucleic acids are treated with formaldehyde and another amine are poorly understood.[10] The modified nucleic acids so far extensively reported have N-CH_2-OH type (methylol) or N-CH_2-N type cross-linked structure.[9a] At least in the case of methylol, the reaction is considered to be reversible,[9a,b] and the structures would be too unstable to account for the mutagenic activity of formaldehyde.[11] Therefore, identification of stable products, if any, would be helpful to understand the molecular basis of mutagenesis or carcinogenesis induced by formaldehyde. In this communication, we report the chemical structure of a stable adduct obtained from the reaction of natural deoxynucleoside and aromatic or aliphatic amines, in the presence of formaldehyde.

It has been well established that formaldehyde reacts rapidly with amino groups of nucleobases and affords hydroxymethyl or imine derivatives. The slower reaction of these adducts then results in the formation of cross-links.[9a] The known reaction of deoxyguanosine with formaldehyde is shown in Scheme 1A.[4a] This reaction occurs at both the monomer and polymer level.[9a] If other amines can participate in this reaction, they would be expected to afford cross-coupling products of deoxynucleoside and amine.

First we attempted to cross-link deoxynucleoside with aniline. A mixture of deoxyribonucleosides (deoxyadenosine, deoxyguanosine, deoxycytidine) (100 mg) and aniline (3 equiv) in methanol (15 mL), was treated with an excess of 35% formaldehyde aqueous solution (30 equiv) and the mixture was stirred for 12 h at room temperature. TLC monitoring showed newly appeared

*Corresponding author. Tel.: +81-3-5841-7847; fax: +81-3-5841-8495; e-mail: hashimot@imcbns.iam.u-tokyo.ac.jp

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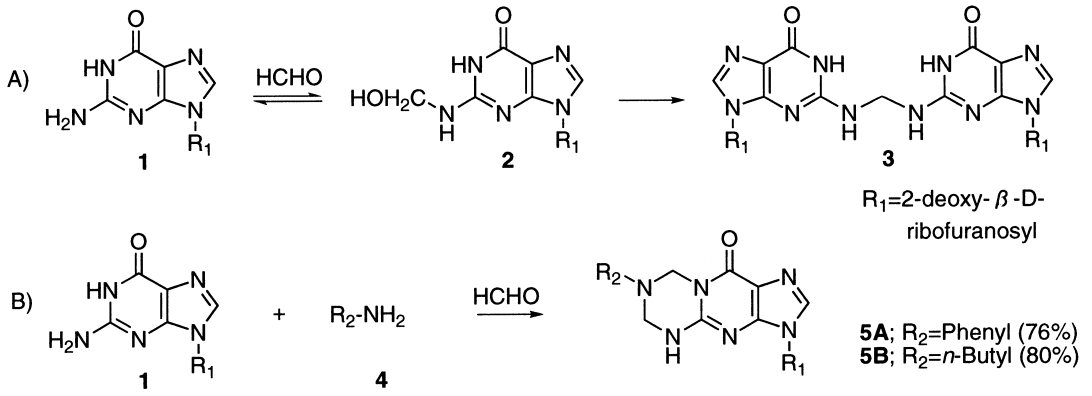


Scheme 1.

spots with R_f values different from those of the cross-linked deoxynucleoside dimers (e.g., **3**) which were prepared elsewhere.[4a] The solution was concentrated under reduced pressure, and the residue was separated by silica gel column chromatography. A cross-linked adduct of deoxyguanosine and aniline was isolated in good yield. In contrast, reaction products derived from either deoxyadenosine or deoxycytidine were decomposed or gave complex mixtures under the conditions of isolation. The structure of the deoxyguanosine adduct produced by formaldehyde-mediated modification with aniline was assigned as **5A** (Scheme 1B).[11]

The proposed structure of **5A** was supported by ^1H, ^{13}C NMR, COSY, HMBC spectra (Fig. 1) and elemental analysis. Deoxyguanosine and aniline were linked by the formation of a six-membered ring. In this structure, the nitrogen of aniline is connected to the 2-amino group and the N-1 position of the guanine moiety via two methylene units which may be derived from 2 equiv of formaldehyde. This cross-linked adduct was isolated in a yield of 76%. Reaction with butyl amine afforded a similar cross-linked adduct, **5B** ($MH^+ = 365.1982$, calcd for $C_{16}H_{25}N_6O_4 = 365.1937$),[11] in a good yield (Scheme 1). Structurally similar compound has been reported as a reaction adduct of synthetic 9-methylguanine and methylamine in the presence of formaldehyde.[12]

It is known that electrophiles can react at various positions of deoxyguanosine, including the exocyclic 2-amino group, endocyclic N-1 and N-7 positions, carbonyl oxygen, and C-8 position. In the Mannich type reaction of purine nucleotides, direct attack of iminium ion is known to occur at the N-7 position.[13] However, in this case, only one cross-linked adduct was isolated as a stable product in a good yield under the very mild experimental conditions. When deoxyguanosine was treated with formaldehyde and methylaniline, no cross-linked product was obtained, though the iminium ion derived from the secondary amine is a stronger electrophile than the imine. In the reaction, the 2-amino group of the deoxyguanosine was observed to be converted very slowly to a methoxymethylamino group. From these observations, it is concluded that formation of the cross-linked adducts, **5A**/**B**, strongly depends on the stability of the six-membered ring, and this would account for the regioselective alkylation of deoxyguanosine with formaldehyde to yield a stable product.

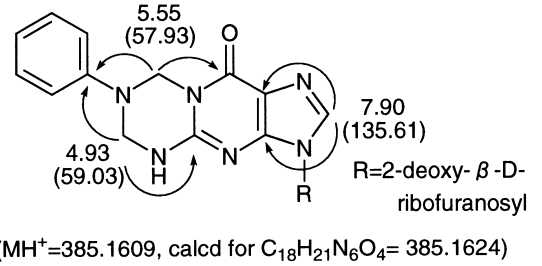
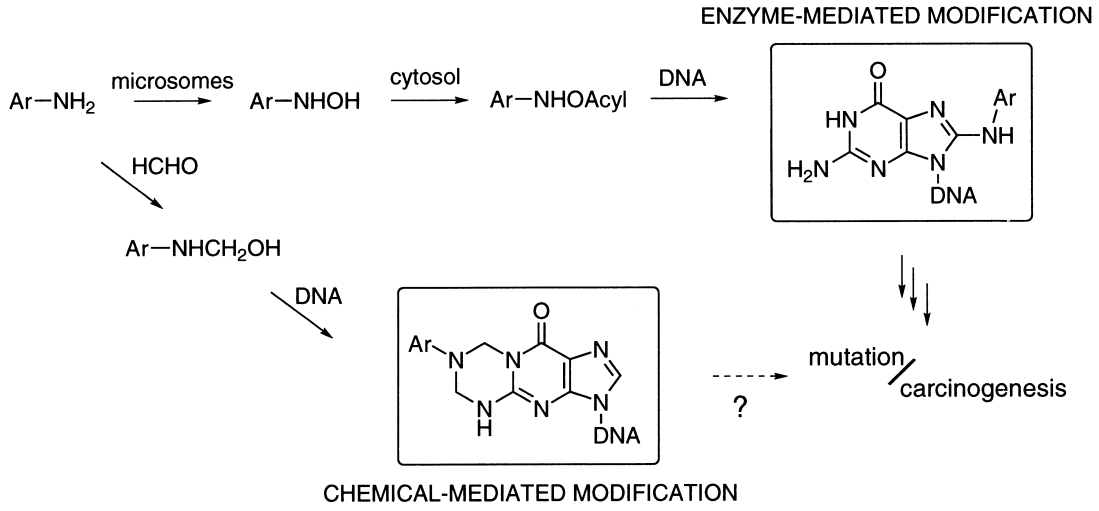


Figure 1. ^1H and ^{13}C NMR (in parentheses) chemical shifts (δ) and HMBC correlations for **5A**.

An aromatic amino moiety is a typical functional group of carcinogens, such as the DNA intercalators naphthylamine and biphenylamine. The modification processes of DNA with carcinogenic aromatic amines have been studied in detail.[14] Carcinogenic aromatic amines are generally metabolized to acyloxyamine derivatives followed by S_N2 reaction mainly at the C-8 position of the guanine moiety (Scheme 2). For these reactions, metabolic (enzymatic) activation (oxidation) of the amino group is mandatory. However, our findings reported here suggest that some primary aromatic or aliphatic amines can alkylate the guanine moiety to afford stable adducts without metabolic activation, in the presence of formaldehyde. Such a condition could be met in vivo in persons exposed to formaldehyde or to environmental chemicals that liberate formaldehyde during their metabolism. Moreover, elevated levels of formaldehyde have been seen in lymphocytes of chronic lymphocytic leukemia than normal lymphocytes.[15] Therefore, our formaldehyde-mediated alkylation of deoxyguanosine may be regarded as a biomimetic model of a modification reaction of DNA.

Recent study on the mechanism of anticancer action of adriamycin revealed that the 3′-amino group of adriamycin cross-links to the 2-amino group of guanine moiety, mediated by formaldehyde.[16] In this case, formaldehyde is produced via hydrogen peroxide oxidation of adriamycin or Tris or spermine.[16b] Adriamycin also induces mammary adenocarcinomas in female Sprague–Dawley rats.[17] This result increases the possibility that formaldehyde-mediated DNA alkylation with other intercalators may be involved in the mutagenic activity elicited by these compounds.



Scheme 2.

In summary, we have identified a novel stable cross-linked adduct of natural deoxyguanosine and primary aromatic or aliphatic amine, formed chemically in the presence of formaldehyde. This reaction appears to be a general reaction of deoxyguanosine and primary amines in the presence of formaldehyde, and we suggest it may be a model of DNA modification. Furthermore, it may be related to formaldehyde-induced mutation, because the modified guanine cannot form base pairing to cytosine. The findings imply that carcinogenic aromatic amines can exert at least a part of their muta-carcinogenic activity without metabolic activation, if formaldehyde is present. Further experiments to assess the biological importance of the reaction are under way.

References and Notes

1. Graves, R. J.; Green, T. *Mutat. Res.* **1996**, *367*, 143.
2. Darl, A. R.; Hadley, W. M. *Toxicol. Appl. Pharmacol.* **1983**, *67*, 200.
3. (a) Schwencke, N.; Ekert, B. *Mutat. Res.* **1978**, *51*, 11. (b) Wilkins, R. J.; MacLeod, H. D. *Mutat. Res.* **1976**, *36*, 11.
4. (a) Chaw, Y. F. M.; Crane, L. E.; Lange, P.; Shapiro, R. *Biochemistry* **1980**, *19*, 5525. (b) Huang, H.; Hopkins, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 9402.
5. (a) Swenberg, J. A.; Kerns, W. D.; Mitchell, R. I.; Gralla, E. J.; Pavkov, K. L. *Cancer Res.* **1980**, *40*, 3398. (b) Kerns, W. D.; Pavkov, K. L.; Donofrio, D. J.; Gralla, E. J.; Swenberg, J. A. *Cancer Res.* **1983**, *43*, 4382.
6. Harman, A. E.; Voigt, J. M.; Frame, S. R.; Bogdanffy, M. S. *Mutat. Res.* **1997**, *380*, 155.
7. (a) Heck, H.d'H.; Casanova, M. *Toxicol. Appl. Pharmacol.* **1999**, *160*, 86. (b) Snyder, R. D.; Van Houten, B. *Mutat. Res.* **1986**, *165*, 21.
8. Merk, O.; Speit, G. *Environ. Mol. Mutagen.* **1998**, *32*, 260.
9. (a) Feldman, M. Y. *Prog. Nucleic Acid Res. Mol. Biol.* **1973**, *13*, 1. (b) McGhee, J. D.; von Hippel, P. H. *Biochemistry* **1975**, *14*, 1281. (c) McGhee, J. D.; von Hippel, P. H. *Biochemistry* **1975**, *14*, 1297. (d) Chang, Y. T.; Loew, G. H. *J. Am. Chem. Soc.* **1994**, *116*, 3548.
10. (a) Siomin, Y. A.; Simonov, V. V.; Poverenny, A. M. *Biochim. Biophys. Acta* **1973**, *331*, 27. (b) Semin, Y. A.; Kolomyitseva, E. N.; Poverennyi, A. M. *Mol. Biol. (U.S.S.R.)* **1974**, *8*, 276. (c) Conrat, H. F.; Olcott, H. *J. Am. Chem. Soc.* **1948**, *70*, 2673.
11. Spectral data are as follows. **5A**: gum; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.14 (1H, ddd, J=13.2, 6.0, 3.0 Hz), 2.47 (1H, m), 3.46 (1H, m), 3.50 (1H, m), 3.76 (1H, dd, J=8.0, 3.0 Hz), 4.29 (1H, s), 4.89 (1H, t, J=6.0 Hz), 4.93 (2H, s), 5.23 (1H, d, J=3.7 Hz), 5.55 (2H, s), 6.03 (1H, dd, J=8.1, 6.0 Hz), 6.92 (1H, t, J=8.0 Hz), 7.06 (2H, d, J=8.0 Hz), 7.26 (2H, t, J=8.0 Hz), 7.90 (1H, s), 8.16 (1H, s); ^{13}C NMR (500 MHz, DMSO-d_6) δ 39.47, 57.93, 59.03, 61.66, 70.69, 82.38, 87.55, 115.83, 117.53, 121.80, 129.44, 135.61, 146.18, 149.24, 150.40, 155.67; HRFABMS calcd for $C_{18}H_{21}N_6O_4$ (MH^+): 385.1624. Found: 385.1609. **5B**: gum; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.85 (3H, t, J=7.4 Hz), 1.28 (2H, tq, J=7.4 Hz), 1.41 (2H, dd, J=7.4 Hz), 2.17 (1H, ddd, J=13.2, 6.0, 3.0 Hz), 2.49 (2H, t, J=7.4 Hz), 2.51 (1H, m), 3.48 (1H, m), 3.52 (1H, m), 3.79 (1H, dd, J=8.0, 3.0 Hz), 4.24 (2H, s), 4.32 (1H, m), 4.89 (2H, s), 4.92 (1H, t, J=6.0 Hz), 5.25 (1H, d, J=3.8 Hz), 6.09 (1H, dd, J=8.0, 6.0 Hz), 7.84 (1H, br), 8.89 (1H, s); ^{13}C NMR (500 MHz, DMSO-d_6) δ 14.56, 20.44, 30.13, 39.50, 49.73, 60.73, 61.41, 62.56, 71.59, 83.25, 88.38, 116.81, 136.16, 150.10, 151.32, 156.97; HRFABMS calcd for $C_{16}H_{25}N_6O_4$ (MH^+): 365.1937. Found: 365.1982.
12. (a) A stable adduct of guanosine-dimer and a product form a reaction using 9-methylguanine have been reported. Kennedy, G.; Slaich, P. K.; Golding, W. P.; Watson, W. P. *Chem. Biol. Interact.* **1996**, *102*, 93. (b) Volkov, V. S.; Poverennyi, A. M.; Sverdlov, E. D. *Bioorg. Khim.* **1987**, *132*, 204.
13. Volkov, V. S. *Biochemistry (Moscow)* **1994**, *59*, 905.
14. (a) Miller, J. A. *Cancer Res.* **1970**, *30*, 559. (b) Tarpley, W. G.; Miller, J. A.; Miller, E. C. *Cancer Res.* **1980**, *40*, 2493. (c) Hashimoto, Y.; Shudo, K.; Okamoto, T. *J. Am. Chem. Soc.* **1982**, *104*, 7636. (d) Hashimoto, Y.; Shudo, K.; Okamoto, T. *Acc. Chem. Res.* **1984**, *17*, 403. (e) Hashimoto, Y.; Kawachi, E.; Shudo, K.; Sekiya, T.; Sugimura, T. *Jpn. J. Cancer Res. (Gann)* **1987**, *78*, 211.
15. Thorndike, J.; Beck, W. S. *Cancer Res.* **1977**, *37*, 1125.
16. (a) Gao, Y. G.; Liaw, Y. C.; Li, Y. k.; van der Marel, G.; van Boom, J. H.; Wang, A. H. J. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4845. (b) Taatjes, D. J.; Gaudiano, G.; Resing, K.; Koch, T. H. *J. Med. Chem.* **1997**, *40*, 1276. (c) Fenick, D. J.; Taatjes, D. J.; Koch, T. H. *J. Med. Chem.* **1997**, *40*, 2452.
17. Bucciarelli, E. *J. Natl. Cancer Inst.* **1981**, *66*, 81.