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[$^{13}C_2$]- Acetaldehyde Promotes Unequivocal Formation of 1,N^2-Propano-2′-deoxyguanosine in Human Cells

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Supporting Information

ABSTRACT: Acetaldehyde is an environmentally widespread genotoxic aldehyde present in tobacco smoke, vehicle exhaust and several food products. Endogenously, acetaldehyde is produced by the metabolic oxidation of ethanol by hepatic NAD-dependent alcohol dehydrogenase and during threonine catabolism. The formation of DNA adducts has been regarded as a critical factor in the mechanisms of acetaldehyde mutagenicity and carcinogenesis. Acetaldehyde reacts with 2′-deoxyguanosine in DNA to form primarily N^2-ethylidene-2′-deoxyguanosine. The subsequent reaction of N^2-ethylidenedGuo with another molecule of acetaldehyde gives rise to 1,N^2-propano-2′-deoxyguanosine (1,N^2-propanodGuo), an adduct also found as a product of the crotonaldehyde reaction with dGuo. However, adducts resulting from the reaction of more than one molecule of acetaldehyde *in vivo* are still controversial. In this study, the unequivocal formation of 1,N^2-propanodGuo by acetaldehyde was assessed in human cells via treatment with [$^{13}C_2$]-acetaldehyde. Detection of labeled 1,N^2-propanodGuo was performed by HPLC/MS/MS. Upon acetaldehyde exposure (703 μM), increased levels of both 1,N^2-etheno-2′-deoxyguanosine (1,N^2-εdGuo), which is produced from α,β-unsaturated aldehydes formed during the lipid peroxidation process, and 1,N^2-propanodGuo were observed. The unequivocal formation of 1,N^2-propanodGuo in cells exposed to this aldehyde can be used to elucidate the mechanisms associated with acetaldehyde exposure and cancer risk.

Acetaldehyde (AA), a proven mutagenic and carcinogenic compound, is formed endogenously as the main product of ethanol oxidation and has been measured in liver and saliva after ingestion of ethanol.[1] In addition, small amounts of AA are produced endogenously during the catabolism of threonine.[2] AA is widespread in the environment, including foods and beverages, cigarette smoke, ethanol metabolism, and fuel combustion (both alcohol and diesel).[3] AA genotoxicity and carcinogenicity are highlighted in populations that are deficient in aldehyde dehydrogenase, a condition which results in a higher risk of esophageal cancer after alcohol consumption compared to populations with the fully active enzyme.[4] The formation of DNA adducts has been regarded as a critical factor in the mechanism of AA toxicity.[5] AA reacts with the 2′-deoxyguanosine (dGuo, **1**, Scheme 1) in DNA to primarily form N^2-ethylidene-2′-deoxyguanosine, an unstable Schiff base (**3**, Scheme 1).[6] The subsequent reaction of adduct **3** with a second molecule of AA leads to the formation of the (6*S*,8*S*) and (6*R*,8*R*) diastereomers of 1,N^2-propanodGuo adducts[7] (**5**, Scheme 1). In addition, 1,N^2-propanodGuo formation is catalyzed by polyamines and histones.[8] 1,N^2-propanodGuo is also formed in the reaction of crotonaldehyde with dGuo and DNA.[9] Regarding its presence in DNA, 1,N^2-propanodGuo adduct exists in equilibrium between the closed and open forms. The open form is favored in double-stranded DNA, whereas the closed form predominates in single-stranded DNA.[10]

1,N^2-PropanodGuo promotes DNA miscoding in human cells, mainly through G→T transversions, and can inhibit DNA synthesis.[11] The ring-opened form can lead to an interstrand cross-link when the adduct is formed in a 5′-CpG sequence.[12] Cross-linking events involving DNA and proteins occur after reaction of the ring-opened free aldehyde with peptides.[13]

The base modification induced by AA is well documented. For example, the reduced form of N^2-ethylidenedGuo (**4**, Scheme 1), the product of the reaction of one molecule of AA with dGuo, has been detected *in vivo*.[14] However, the formation of 1,N^2-propanodGuo from AA *in vivo* is controversial because it requires two successive reactions of AA. Its presence is mainly credited to the reaction of dGuo with the crotonaldehyde generated during lipid peroxidation (Scheme 1); however, the formation of protein adducts involving more than one molecule of AA was observed in patients with alcohol-induced liver disease.[15]

Additionally, epoxidized α,β-unsaturated aldehydes, which are end products of lipid peroxidation, can generate ethano or etheno derivatives upon reaction with DNA.[16] 1,N^2-εdGuo (**2**, Scheme 1), for example, is derived from the reaction between *trans,trans*-2,4-decadienal (DDE) and dGuo[17] (Scheme 1). Some of these DNA lesions have proved to be highly mutagenic[18] and are considered possible pathways for oxidative stress-related carcinogenesis.

In the present study, the formation of exocyclic DNA adducts was investigated in cells treated with AA. Because of the volatility of AA, its actual concentration in the cell medium was estimated through conjugation with 2,4-dinitrophenylhydrazine (DNPH). The resulting hydrazones were analyzed by HPLC with UV detection (λ = 360 nm). The effective concentrations in the stock solutions made in water and the concentrations measured in the culture cell media presented high AA evaporation rates. Nevertheless, AA can also react promptly with cell media

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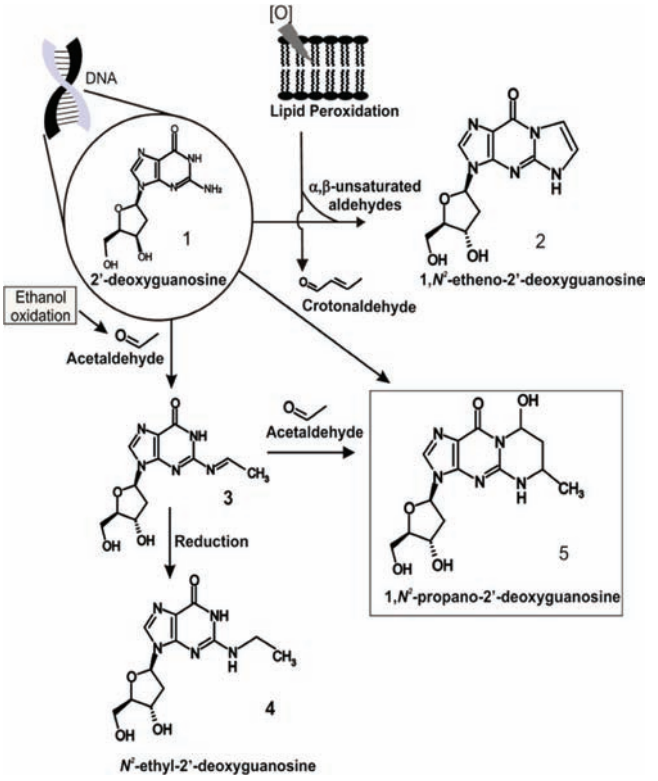
Scheme 1. Representative Formation of DNA Adducts from the Lipid Peroxidation End Products, 1,N^2-εdGuo (2) and 1,N^2-PropanodGuo (5), and from Acetaldehyde, N^2-EthylidenedGuo (3) and 1,N^2-PropanodGuo (5)



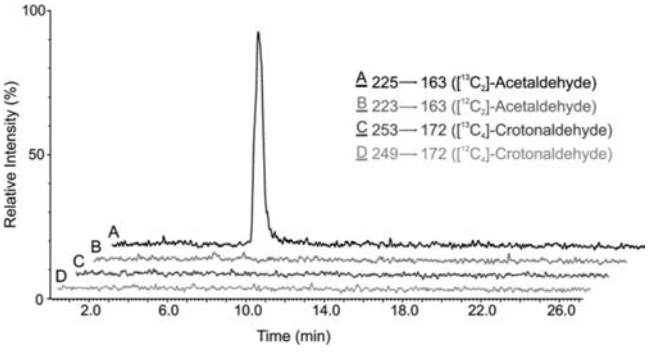


Figure 1. Analysis of DNPH derivatives in cell supernatant. Cells were treated with 10 mM [$^{13}C_2$]-acetaldehyde for 3 h; subsequently, cell media aliquots were taken and derivatized (1:1) with 20 mM DNPH. HPLC/ESI/MS/MS analyses, carried out in the negative mode of the corresponding derivatives, are the following: (A) [$^{13}C_2$]-Acetaldehyde (225/163). (B) [$^{12}C_2$]-Acetaldehyde (223/163). (C) [$^{13}C_4$]-Crotonaldehyde (253/172). (D) [$^{12}C_4$]-Crotonaldehyde (249/172).

components and with protein from the cell membrane, therefore decreasing its availability (Supporting Information).

In order to verify if crotonaldehyde arising from aldol condensation is formed in the cell media, cells were treated with 703 μM [$^{13}C_2$]-AA for 3 h, and subsequently, cell media aliquots were taken and derivatized (1:1) with 20 mM DNPH. The resulting hydrazones were analyzed by HPLC/ESI/MS/MS.

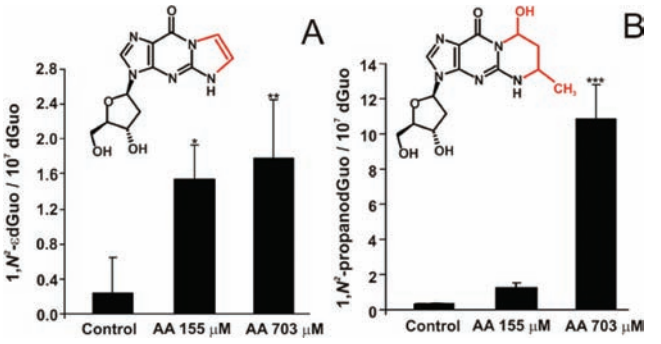


Figure 2. Quantification by HPLC/MS/MS of 1,N^2-εdGuo/10^7dGuo (A) and 1,N^2-propanodGuo/10^7dGuo (B) in 100 μg of cell-extracted DNA after treatment with AA at 155 and 703 μM. (*) $p < 0.05$ compared to control; (**) $p < 0.01$ compared to control, (***) $p < 0.001$ compared to control and 155 μ M.

Figure 1 shows that crotonaldehyde was not detected under our experimental conditions.

Exocyclic DNA adducts were analyzed by HPLC/MS/MS using an API 4000 QTRAP mass spectrometer (Applied Biosystems) as described by Garcia et al.[19] The adducts were detected by multiple reaction monitoring (MRM). Briefly, the DNA hydrolysates containing 100 fmol of internal standards were injected into the HPLC/ESI/MS/MS system. The m/z 292→176 (1,N^2-εdGuo), 297→181 ([$^{15}N_5$]1,N^2-εdGuo), 338→222 (1,N^2-propanodGuo) and 343→227 ([$^{15}N_5$]1,N^2-propanodGuo) transitions were monitored with a dwell time of 280 ms. All other mass spectrometer parameters were adjusted for acquisition of the best $[M + H]^+/[M + H - 2\text{-D-}erythro\text{-pentose}]^+$ transition.

IMR-90 cells were treated with AA (155 μM or 703 μM) for 3 h. Cell viability as measured by the MTT assay was higher than 70% after AA treatment (data not shown). DNA was extracted, hydrolyzed, and analyzed as already described, with minor modifications.[19] Using this highly sensitive methodology, a significant increase in the levels of both 1,N^2-propanodGuo and 1,N^2-εdGuo was observed (Figure 2).

In the present experimental approach, 1,N^2-εdGuo could only arise from aldehydes formed endogenously, whereas 1,N^2-propanodGuo could arise from exogenous AA or endogenous aldehydes produced during the lipid peroxidation process. In fact, AA was able to induce lipid peroxidation in cells, as measured by malondialdehyde production (Supporting Information).

According to the DNA adduct quantification (Figure 2), after treatment with 703 μM of aldehyde, the increase in levels of etheno adducts compared to control cells was lower (about 5 times, Figure 2A) than the increase in propano adducts (20 times, Figure 2B). Moreover, formation of the propano adduct seems to present a more direct correlation with increasing AA concentration than does formation of the etheno adduct, supporting the relevance of this lesion as biomarker of AA exposure. In contrast, upon treatment with 155 μM aldehyde, the relative increase in levels of the etheno adduct was higher than that observed for the propano adduct, showing that adducts are preferentially formed via the lipid peroxidation pathway at this concentration.

The unequivocal formation of 1,N^2-propanodGuo in a two-step reaction with exogenously added AA was assessed by treating cells with [$^{13}C_2$]-AA and detecting the labeled adduct

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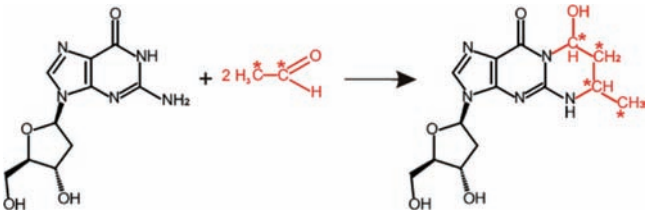
Scheme 2. Reaction of dGuo with Two Molecules of [$^{13}C_2$]-Acetaldehyde



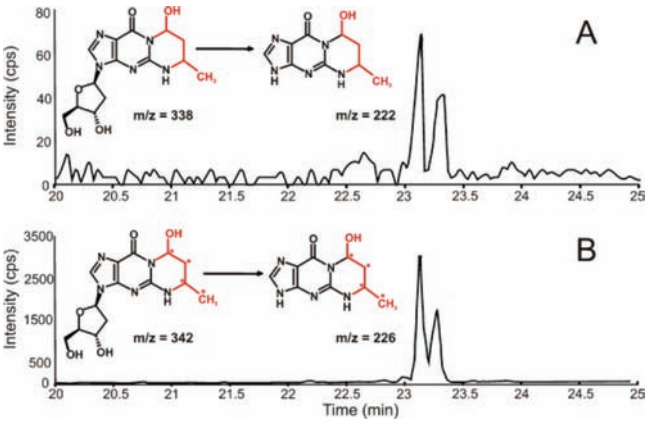


Figure 3. Representative chromatogram obtained by HPLC/MS/MS of hydrolyzed DNA from cells treated with 703 μM [$^{13}C_2$]-acetaldehyde. (A) Chromatographic detection of normal basal levels of 1,N^2-propanodGuo (m/z 338/222). (B) Detection of [$^{13}C_4$]-labeled 1,N^2-propanodGuo (m/z 342/226) by the addition of two [$^{13}C_2$]-AA.

(Scheme 2). The adducts were detected by HPLC/MS/MS using an API 4000 QTRAP mass spectrometer (Applied Biosystems) in the MRM mode. Briefly, the DNA hydrolysates were injected into the HPLC/ESI/MS/MS system. Then, the m/z 338→222 (1,N^2-propanodGuo) and 342→226 ([$^{13}C_4$]1,N^2-propanodGuo) transitions were monitored. The formation of a molecule 4 amu over the expected weight of 1,N^2-propanodGuo confirmed that two molecules of labeled AA had been incorporated. In order to exclude possible artifactual adduct formation due to carryover of AA during DNA extraction, 1,N^2-propanodGuo was measured in DNA extracted immediately (0 h) and 3 h after AA treatment. 1,N^2-PropanodGuo formation at 0 h was not detected in our analytical platform (limit of detection 10 fmol) (Supporting Information). Thus, the resulting 1,N^2-propanodGuo molecule was due to treatment with labeled AA and was not due to nonspecific lipid peroxidation product or artifactual formation (Scheme 2 and Figure 3).

In addition, the reduced form of the N^2-ethylidenedGuo was quantified in DNA from cells treated with 703 μM of [$^{13}C_2$]AA using a newly developed methodology, as described in Materials and Methods (Supporting Information). The levels of such adducts were comparable to the level of 1,N^2-propanodGuo. This finding is somewhat intriguing due to the fact that the formation of 1,N^2-propanodGuo involves the sequential additions of two molecules of AA. Nevertheless, a similar result had already been reported by Inagaki et al.[20] in cells exposed to high concentrations of AA. It was discussed that for low AA concentrations the formation of 1,N^2-propanodGuo should be unfavorable and N^2-ethylidenedGuo should be formed more promptly. Therefore, it is important to state that N^2-ethylidenedGuo would be the main adduct formed under human exposure conditions. Nevertheless, more studies are warranted in order to fully comprehend this phenomenon.

In conclusion, the application of our highly sensitive HPLC/MS/MS method, in combination with [$^{13}C_2$]-AA, showed direct and unequivocal AA-induced formation of 1,N^2-propanodGuo in living cells. In addition, higher levels of endogenously generated 1,N^2-εdGuo could be detected after AA exposure. The adducts detected here could play a role in the genotoxicity of AA, an ubiquitous environmental pollutant and alcohol metabolite.

■ ASSOCIATED CONTENT

Supporting Information. Experimental section and Results: Determination of acetaldehyde concentrations, Malondialdehyde determination and Assessment of [$^{13}C_4$]1,N^2-propanodGuo generated by AA carryover during DNA extraction. This material is available free of charge via the Internet at http://pubs.acs.org

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■ REFERENCES

(1) (a) Visapaa, J. P.; Gotte, K.; Benesova, M.; Li, J.; Homann, N.; Conradt, C.; Inoue, H.; Tisch, M.; Horrmann, K.; Vakevainen, S.; Salaspuro, M.; Seitz, H. K. *Gut.* **2004**, *53*, 871–876. (b) Yokoyama, A.; Tsutsumi, E.; Imazeki, H.; Suwa, Y.; Nakamura, C.; Mizukami, T.; Yokoyama, T. *Alcohol.: Clin. Exp. Res.* **2008**, *32*, 1607–1614.

(2) Ogawa, H.; Gomi, T.; Fujioka, M. *Int. J. Biochem. Cell Biol.* **2000**, *32*, 289–301.

(3) (a) *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*; International Agency for Research on Cancer: Lyon, France, 1985; Vol. *36*, pp 101−132. (b) *Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*; International Agency for Research on Cancer: Lyon, France, 1999; Vol. 71, pp 319−335. (c) Lachenmeier, D. W.; Sohnius, E. M. *Food Chem. Toxicol.* **2008**, *46*, 2903–2911.

(4) (a) Matsuda, T.; Yabushita, H.; Kanaly, R. A.; Shibutani, S.; Yokoyama, A. *Chem. Res. Toxicol.* **2006**, *19*, 1374–1378. (b) Ogawa, M.; Oyama, T.; Isse, T.; Saito, K.; Tomigahara, Y.; Endo, Y.; Kawamoto, T. *Toxicol. Lett.* **2007**, *168*, 148–154.

(5) Brooks, P. J.; Theruvathu, J. A. *Alcohol* **2005**, *35*, 187–193.

(6) Wang, M.; McIntee, E. J.; Cheng, G.; Shi, Y.; Villalta, P. W.; Hecht, S. S. *Chem. Res. Toxicol.* **2000**, *13*, 1149–1157.

(7) Hecht, S. S.; McIntee, E. J.; Wang, M. *Toxicology* **2001**, *166*, 31–36.

(8) (a) Sako, M.; Inagaki, S.; Esaka, Y.; Deyashiki, Y. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 3497–3498. (b) Theruvathu, J. A.; Jaruga, P.; Nath, R. G.; Dizdaroglu, M.; Brooks, P. J. *Nucleic Acids Res.* **2005**, *33*, 3513–3520.

(9) (a) Chung, F. L.; Young, R.; Hecht, S. S. *Cancer Res.* **1984**, *44*, 990–995. (b) Wang, M.; Upadhyaya, P.; Dinh, T..T.; Bonilla, L. E; Hecht, S. S. *Chem. Res. Toxicol.* **1998**, *11*, 1567–1573.
(10) Kurtz, A. J.; Lloyd, R. S. *J. Biol. Chem.* **2003**, *278*, 5970–5976.
(11) Stein, S.; Lao, Y.; Yang, I. Y.; Hecht, S. S.; Moriya, M. *Mutat. Res.* **2006**, *608*, 1–7.
(12) Kozekov, I. D.; Nechev, L. V.; Moseley, M. S.; Harris, C. M.; Rizzo, C. J.; Stone, M. P.; Harris, T. M. *J. Am. Chem. Soc.* **2003**, *125*, 50–61.
(13) Kurtz, A. J.; Lloyd, R. S. *J. Biol. Chem.* **2003**, *278*, 5970–5976.
(14) Chen, L.; Wang, M.; Villalta, P. W.; Luo, X.; Feuer, R.; Jensen, J.; Hatsukami, D. K.; Hecht, S. S. *Chem. Res. Toxicol.* **2007**, *20*, 108–113.
(15) Rolla, R.; Vay, D.; Mottaran, E.; Parodi, M.; Traverso, N.; Arico, S.; Sartori, M.; Bellomo, G.; Klassen, L. W.; Thiele, G. M.; Tuma, D. J.; Albano, E. *Hepatology* **2000**, *31*, 878–884.
(16) (a) Medeiros, M. H. G. *Chem. Res. Toxicol.* **2009**, *22*, 419–425. (b) Blair, I. *J. Biol. Chem.* **2008**, *283*, 15545–15549. (c) Nair, U.; Bartsch, H.; Nair, J. *Free Radical Biol. Med.* **2007**, *43*, 1109–1120. (d) Golding, B. T.; Slaich, P. K.; Kennedy, G.; Bleasdale, C.; Watson, W. P. *Chem. Res. Toxicol.* **1996**, *9*, 147–157.
(17) Loureiro, A. P. M.; Di Mascio, P.; Gomes, O. F.; Medeiros, M. H. G. *Chem. Res. Toxicol.* **2000**, *13*, 601–609.
(18) (a) Basu, A. K.; Wood, M. L.; Niedernhofer, L. J.; Ramos, L. A.; Essigmann, J. M. *Biochemistry* **1993**, *32*, 12793–12801. (b) Pandya, G. A.; Moriya, M. *Biochemistry* **1996**, *35*, 11487–11492. (c) Palejwala, V. A.; Rzepka, R. W.; Simha, D.; Humayun, M. Z. *Biochemistry* **1993**, *32*, 4105–4111. (d) Moriya, M.; Zhang, W.; Johnson, F.; Grollman, A. P. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 11899–11903. (e) Cheng, K. C.; Preston, B. D.; Cahill, D. S.; Dosanjh, M. K.; Singer, B.; Loeb, L. A. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 9974–9978.
(19) Garcia, C. C.M.; Freitas, F. P.; Di Mascio, P.; Medeiros, M. H.G. *Chem. Res. Toxicol.* **2010**, *23*, 1245–1255.
(20) Inagaki, S.; Esaka, Y.; Deyashiki, Y.; Sako, M.; Goto, M. *J. Chromatogr., Sect. A* **2003**, *987*, 341–347.
(21) Poli, G; Dianzani, M. U.; Cheeseman, K. H.; Slater, T. F.; Lang, J.; Esterbauer, H. *Biochem. J.* **1985**, *227*, 629–638.
(22) Wang, L.; Hirayasu, K.; Ishizawa, M.; Kobayashi, Y. *Nucleic Acids Res.* **1994**, *22*, 1774–1775.
(23) Loureiro, A. P. M.; Marques, S. A.; Garcia, C. C. M.; Di Mascio, P.; Medeiros, M. H. G. *Chem. Res. Toxicol.* **2002**, *15*, 1302–1308.
(24) Sako, M.; Kawada, H.; Hirota, K. *J. Org. Chem.* **1999**, *64*, 5719–5721.
(25) Tatum, V. L.; Ghangchit, C.; Chow, C. K. *Lipids* **1990**, *25*, 226–229.
(26) Bradford, M. M. *Anal. Biochem.* **1976**, *72*, 248–254.