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Identification of an Acetaldehyde Adduct in Human Liver DNA and Quantitation as N^2-Ethyldeoxyguanosine

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Abstract

Acetaldehyde, a ubiquitous mutagen and carcinogen, could be involved in human cancer etiology. Since DNA adducts are important in carcinogenesis, we have used liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) to explore the presence in human liver DNA of the major acetaldehyde DNA adduct, N^2-ethylidenedeoxyguanosine (**1**). DNA was isolated and enzymatically hydrolyzed in the presence of $NaBH_3CN$, which quantitatively converts adduct **1** to N^2-ethyldeoxyguanosine (**2**). [$^{15}N_5$]N^2-Ethyl-dGuo was synthesized and used as internal standard. Adduct **2** was enriched from the hydrolysate by solid phase extraction and analyzed by LC-ESI-MS/MS. Clear peaks were observed for adduct **2** in analyses of human liver DNA, calf thymus DNA, and rat liver DNA. These peaks were not observed, or were much smaller, when the $NaBH_3CN$ step was omitted. When the DNA was subjected to neutral thermal hydrolysis prior to $NaBH_3CN$ treatment, adduct **2** was not observed. Control experiments using [$^{13}C_2$]acetaldehyde demonstrated that adducts **1** and **2** were not formed as artifacts during DNA isolation and analysis. These results strongly indicate that adduct **1** is present in human liver DNA and demonstrate that it can be quantified as adduct **2**. Levels of adduct **2** measured in 12 human liver samples were 534 ± 245 fmol/μmol dGuo (mean ± S.D.). The results of this study establish the presence of an acetaldehyde adduct in human liver DNA and suggest that it is a commonly occurring endogenous DNA adduct.

Introduction

Acetaldehyde is ubiquitous in the human environment (1). It is the major metabolite of ethanol. It occurs widely in food, being found in many common fruits and vegetables, as well as cooked meat. It is a common environmental combustion product, with an estimated 99 million pounds emitted in the U.S. from residential burning, and is found in gasoline exhaust and diesel exhaust. It is one of the most prevalent carcinogens in cigarette smoke, with levels of about 1 mg/cigarette, 100,000 times as great as benzo[*a*]pyrene. There are many potential occupational exposures to acetaldehyde as well (1).

Treatment of rats with acetaldehyde by inhalation caused adenocarcinoma and squamous cell carcinoma of the nasal mucosa (2,3). In hamsters, inhalation of acetaldehyde produced laryngeal carcinoma and enhanced the occurrence of respiratory tract tumors caused by intra-tracheal instillation of benzo[*a*]pyrene (2,3). Acetaldehyde is genotoxic, causing mutations, sister chromatid exchanges, micronuclei and aneuploidy in cultured mammalian cells, as well as gene mutations in bacteria (2,3). Some studies indicate that acetaldehyde may be involved in alcohol-related cancer in humans (3). Acetaldehyde is reasonably anticipated to be a human carcinogen by the U.S. Dept. of Health and Human Services

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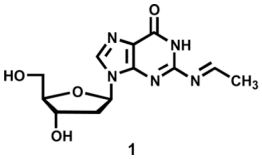
(USDHHS), and is possibly carcinogenic in humans according to the International Agency for Research on Cancer (IARC) (1,3). Acetaldehyde is likely to play a significant role in cancer induction by alcohol consumption, classified as a known human carcinogen by both IARC and USDHHS (1,4).

DNA adducts are critical in carcinogenesis. Quantitation of acetaldehyde-DNA adducts in human tissues would be important in assessing its potential role as a human carcinogen. The major DNA adduct of acetaldehyde formed upon reaction with DNA in vitro is N^2-ethylidene-dGuo (**1**), which is quite stable in DNA, but quickly decomposes at the nucleoside level (5,6). Treatment of DNA containing **1** with $NaBH_3CN$ produces N^2-ethyl-dGuo (**2**), which is stable (5). Fang and Vaca developed a ^{32}P-postlabelling method for the analysis of **2**, and detected this adduct in peripheral white blood cells of alcohol abusers (7,8). Matsuda et al detected **2** in human urine by liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) (9). Neither of these studies used $NaBH_3CN$ reduction during the analysis, and **2** was presumed to have arisen by endogenous reduction of **1**. Inagaki et al reported an LC-ESI-MS method for analysis of **2** but applied it only in vitro (10). In the present study, we have developed a LC-ESI-MS/MS method for the quantitation of **2** in DNA samples treated with $NaBH_3CN$, and have applied it to the analysis of DNA from human liver.



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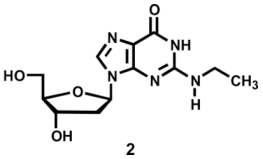

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Experimental Section

HPLC-UV Analysis

This was carried out using Waters Associates (Milford, MA) instruments equipped with a UV detector (Shimadzu Scientific Instruments, Columbia, MD) operated at 254 nm. System 1 used two 4.6 mm × 25 cm C18 reversed-phase columns with isocratic elution by 5% CH_3CN for 10 min and then a gradient from 5 to 30% CH_3CN over the course of 50 min at a flow rate of 0.5 mL/min. This system was used for the purification of [$^{15}N_5$]dGTP and adduct **2**. System 2 used a 4.6 mm × 25 cm Luna 5 μm C18(2) column (Phenomenex, Torrance, CA) with a gradient from 5 to 40% CH_3OH over the course of 35 min at a flow rate of 0.7 mL/min. This system was used for the analysis of dGuo. System 3 used two 4.6 mm × 25 cm Supelcosil LC 18-DB columns (Supelco, Bellefonte, PA) with isocratic elution by 5% CH_3OH in 10 mM phosphate buffer, pH 7, for 10 min, then a gradient from 5 to 25% CH_3OH in 60 min at a flow rate of 1 mL/min. This system was used for analysis of adduct **2** and [$^{15}N_5$]N^2-ethyl-dGuo ([$^{15}N_5$]**2**).

Chemicals and Enzymes

[$^{15}N_5$]dGTP was obtained from Spectra Stable Isotopes (Columbia, MD). [$^{13}C_2$]Acetaldehyde was procured from Cambridge Isotope Laboratories (Andover, MA). Ethanol was obtained from AAPER Alcohol and Chemical Co. (Shelbyville, Ky). 2-Propanol was purchased from Acros Organics (Morris Plains, NJ). Puregene DNA purification solutions were purchased from Gentra Systems (Minneapolis, MN). Calf thymus DNA, DNase I and phosphodiesterase I were obtained from Sigma-Aldrich (St. Louis, MO). Alkaline phosphatase (from calf intestine) was obtained from Roche Diagnostics Corporation (Indianapolis, IN). All other chemicals were purchased from Sigma-Aldrich.

[$^{15}N_5$]dGuo

[$^{15}N_5$]dGTP (6.6 mg) was dissolved in 1.32 mL of 50 mM Tris-HCl/5mM $MgCl_2$ buffer (pH 9), and 825 units of alkaline phosphatase was added. The mixture was incubated for 3 h at 37 °C. The product was collected from HPLC system 1 (24 to 31 min) and concentrated to dryness.

N^2-Ethyl-dGuo(2) and [$^{15}N_5$]2

Adduct **2** was prepared as described from dGuo and CH_3CHO (11), except that solid phase extraction on a 6 mL C18 Sep-pak (Waters) was used for purification instead of HPLC. The Sep-pak was washed with 0–30% CH_3OH in H_2O. Adduct **2** eluted in the 20% CH_3OH fraction: UV λ_{max} (ε) 254 (15,000); purity >99% by HPLC-UV (System 3). [$^{15}N_5$]**2** was prepared the same way from [$^{15}N_5$]dGuo and was purified by HPLC system 1: MS *m/z* (rel int) 301 $(M + H)^+$ (100), 185 $(BH)^+$ (22); purity > 99% by HPLC-UV (System 3); quantified by UV at 254 nm. The content of adduct **2** in [$^{15}N_5$]**2**, as determined by LC-MS, was less than 0.5%.

Human Liver Samples

This study was approved by the University of Minnesota Research Subjects' Protection Programs Institutional Review Board Human Subjects Committee. Ten samples were obtained from The Cancer Center Tissue Procurement Facility. The samples were histologically confirmed normal liver tissue obtained at surgery and immediately frozen in liquid N_2. Two samples were obtained courtesy of Professor F. Peter Guengerich, Vanderbilt University.

DNA Isolation

This was performed as described in the "DNA Purification from 1 g Animal Tissue" protocol (Gentra Systems, Minneapolis, MN) with several modifications. Human liver tissue samples (0.5 g) were homogenized with 15 mL volumes of Puregene cell lysis solution (PCLS). For experiments using $NaBH_3CN$ and/or [$^{13}C_2$]acetaldehyde, the tissue samples were initially homogenized with 10 mL PCLS containing 150 mM $NaBH_3CN$, followed by an additional 5 mL PCLS containing 5.7 mM [$^{13}C_2$]acetaldehyde. For experiments using $NaBH_3CN$, the isopropanol, Tris-EDTA, ethanol and 70% ethanol solutions contained 100 mM $NaBH_3CN$. The addition of $NaBH_3CN$ during the homogenization and DNA isolation steps is considered prudent to avoid artifact formation. After the DNA had been precipitated and washed with 70% ethanol as described in the Gentra Systems protocol, it was dissolved in 4 mL of 10 mM Tris-HCl/5 mM EDTA buffer at pH 7 and the mixture was extracted twice with 4 mL of $CHCl_3$ containing 4% isoamyl alcohol. The DNA was precipitated from the aqueous phase by addition of 0.4 mL of 5 M NaCl and 8 mL ice-cold ethanol, washed 3 times with 3 mL of 70% ethanol, 3 times with 3 mL of 100% ethanol, and dried with a stream of N_2. The purity of the DNA was determined by measuring its UV absorption at 230, 260, and 280 nm. The ratios A260:230 and A260:280 were greater than 2.0 and 1.7, respectively. DNA from the livers of 12 male Wistar rats (337 ± 16 g) that had been maintained on tap water and NIH-07 diet was similarly isolated.

Analysis of DNA for N^2-Ethyl-dGuo(2)

For enzyme hydrolysis, DNA (0.1–1.6 mg) was dissolved in 900 uL of 10 mM Tris-HCl/5 mM $MgCl_2$ buffer containing [$^{15}N_5$]**2** (243 fmol) and $NaBH_3CN$ (31 mg). The pH was adjusted to 7.2 with HCl. The DNA was initially digested overnight at room temperature with 1,300 units of DNase I (type II, from bovine pancreas). Then to the resulting mixture were added 1,300 additional units of DNase I, 0.07 unit of phosphodiesterase I (type II, from *Crotalus adamanteus* venom), and 750 units of alkaline phosphatase. The mixture was

incubated at 37 °C for 60 min and then allowed to stand overnight at room temperature. Enzymes were removed by centrifugation using a centrifree MPS device (MW cutoff of 30 000; Amicon, Beverly, MA). The hydrolysate, after removal of a 10 uL aliquot for dGuo analysis, was desalted and purified using a solid-phase extraction cartridge (Strata-X 33 μm, 30 mg/1 mL (Phenomenex, Torrance, CA). After adjustment of the hydrolysate to pH 7 (to ensure protonation of the N-1 nitrogen of **2** which has a pK_a of 9.4) with 300 μL of 3 M Tris-HCl (pH 7), it was applied to the Strata-X cartridge. The cartridge was washed with 1 mL H_2O and 1 mL 10% aqueous CH_3OH. Adduct **2** was eluted with 1 mL 70% CH_3OH. The eluants were evaporated to dryness, dissolved in 1 mL H_2O, and purified using a mixed-mode, anion-exchange and reversed-phase extraction cartridge (Oasis MAX, 500 mg/ cartridge, Waters) employing a 2-dimensional elution profile. The pH of the sample was adjusted to > 12 (to form the anion of **2**) by the addition of 300 μL of 0.2 N NaOH, and it was applied to the Oasis MAX cartridge which had been equilibrated with 0.2 N NaOH. The cartridge was washed with 10 mL 0.01 N NaOH, 12 mL 0.01 N KOH in CH_3OH, 2 mL H_2O, 8 mL of 1 M ammonium acetate (pH 6.8), 2 mL H_2O, and 6 mL 10% CH_3OH in H_2O. Adduct **2** was eluted with 6 mL 70% CH_3OH, and the solution was evaporated to dryness. The residue was dissolved in 20 μL H_2O, and 6 uL aliquots were analyzed by LC-ESI-MS/MS.

The analysis was carried out with an Agilent 1100 capillary flow HPLC (Agilent Technologies, Palo Alto, CA) with a 250 mm × 0.5 mm 5 μm particle size C18 column (Agilent Zorbax SB-C18) and either a Finnigan Quantum Ultra AM or a Discovery Max (Thermoelectron, San Jose, CA) triple quadrupole mass spectrometer. The solvent elution program was a 10 μL/min gradient from 5% to 40% CH_3OH in 35 min at 30 °C. The ESI source was set in the positive ion mode as follows: voltage, 3.7 kV; current, 3 μA; and heated ion transfer tube, 275 °C. Adducts were quantified by MS/MS using the selected reaction monitoring (SRM) mode, with ion transitions of *m/z* 296 → *m/z* 180 (adduct **2**), *m/z* 298 → *m/z* 182 [$^{13}C_2$]**2**, and *m/z* 301 → *m/z* 185 [$^{15}N_5$]**2**. The collision energy was 12 eV, and the Ar collision gas pressure was 1.0 mTorr.

Calibration curves were constructed before each analysis using standard solutions of 2 and [$^{15}N_5$]2 which were prepared freshly each time and stored at −20 °C until use. A constant amount of [$^{15}N_5$]2 (500 fmol) was mixed with differing amounts of 2 (10–500 fmol) and analyzed by LC-ESI-MS/MS.

Neutral thermal hydrolysis experiments

DNA (1–2 mg) was dissolved in 750 μL of 10 mM Tris-HCl/5 mM $MgCl_2$ buffer (pH 7.0) and heated at 100 °C for 1 h prior to the addition of the internal standard and analysis as above.

Results

The internal standard for our analysis was [$^{15}N_5$]*N*^{2}-ethyl-dGuo ([$^{15}N_5$]**2**), prepared by reaction of acetaldehyde with [$^{15}N_5$]dGuo in the presence of $NaBH_3CN$. It was characterized by HPLC, LC-ESI-MS, UV, and comparison to *N*^{2}-ethyl-dGuo (**2**). LC-ESI-MS/MS-SRM chromatograms of adduct **2** (10 fmol) and [$^{15}N_5$]**2** (100 fmol) are illustrated in Figure 1. The transitions monitored were *m/z* 296 → *m/z* 180 [$(M + H)^+ \rightarrow (BH)^+$] for adduct **2** and *m/z* 301 → *m/z* 185 for [$^{15}N_5$]**2**. A calibration curve for 2 and [$^{15}N_5$]**2** is shown in Figure 2.

DNA was enzymatically hydrolyzed in the presence of $NaBH_3CN$. Control experiments demonstrated that $NaBH_3CN$ had no effect on the efficiency of DNA hydrolysis and that, under our conditions, adduct **1** was quantitatively converted to adduct **2** (5). Adduct **2** was

enriched from the hydrolysate by solid phase extraction on a mixed mode anion exchange reversed-phase cartridge. The eluant containing adduct **2** was then analyzed by LC-ESI-MS/MS-SRM.

Experiments were carried out with and without $NaBH_3CN$ or heat treatment of the DNA to explore the identity of the adduct in DNA. These results are illustrated in Figure 3A, B for calf thymus DNA. Panel A demonstrates the presence of adduct **2** in calf thymus DNA which had been treated with $NaBH_3CN$ and analyzed as described above. Panel B shows that this peak was not observed to a significant extent when $NaBH_3CN$ was omitted. This is consistent with the instability of N^2-ethylidene-dGuo (**1**) at 37 °C at the nucleoside level, although its half-life is approximately 24 h in DNA at 37 °C (5,6). The analyte peak was not observed when the DNA sample was heated (100 °C, 1h) prior to $NaBH_3CN$ treatment. This is consistent with the thermal instability of adduct **1** in DNA at 100 °C, although adduct **2** is completely stable under these conditions (5). Similar results were obtained upon analysis of DNA isolated from human liver (Figure 3C, D) and rat liver (data not shown). Taken together, these results indicate that adduct **1** is present in calf thymus DNA, human liver DNA, and rat liver DNA, and is converted to adduct **2** upon $NaBH_3CN$ treatment of the DNA.

We considered the possibility that adduct **1** could be formed in human liver DNA as an artifact during analysis. This was possible because intracellular acetaldehyde could be released during tissue homogenization and react with DNA. We tested this by adding [$^{13}C_2$]acetaldehyde to the cell lysis solution used at the beginning of the experiment for tissue homogenization, and either added or did not add $NaBH_3CN$ at this step. In all other respects, the analytical procedure was identical to that described above, e.g. $NaBH_3CN$ was still added to all samples during DNA hydrolysis. The DNA was analyzed for adduct **2** and [$^{13}C_2$]N^2-ethyl-dGuo ([$^{13}C_2$]**2**). The results are summarized in Table 1 for two human liver samples. The results demonstrate that, in the absence of $NaBH_3CN$ added at the beginning of the procedure, artifact formation is possible because a substantial amount of [$^{13}C_2$]**2**, 4050 fmol/μmol dGuo, was formed. The amount of unlabelled adduct **2** observed in this analysis was 549 fmol/μmol dGuo. When $NaBH_3CN$ was added at the beginning of the analysis, more than 99% of the formation of [$^{13}C_2$]**2** was prevented, but the level of unlabelled adduct **2** was 501 fmol/μmol dGuo, about the same as observed in the absence of $NaBH_3CN$ addition at the beginning of the analysis. The same results were observed for the second human liver sample (Table 1). These results clearly demonstrate that unlabelled **1** and **2** did not form as artifacts during tissue homogenization and DNA analysis because if they had, the levels of adduct **2** would have been reduced to the same extent as those of [$^{13}C_2$]**2** by $NaBH_3CN$ treatment.

Acetaldehyde contamination of solvents used for DNA isolation was also possible. When we added 200 ppm acetaldehyde to the ethanol used for precipitation of DNA, levels of adduct **2** increased, but no increase was observed at 2 ppm or 20 ppm addition (data not shown). To explore this possibility further, we compared adduct levels in DNA isolated using ethanol or isopropanol for precipitation, when these solvents were either treated with $NaBH_4$ before use to reduce any acetaldehyde that might have been present, or used directly. The results demonstrated that $NaBH_4$ treatment of these solvents had no effect on levels of adduct **2** in DNA. Therefore, we concluded that artifact formation did not occur during DNA precipitation.

Accuracy and precision were determined by adding **2** to rat liver DNA and analyzing multiple samples. The results are summarized in Table 2, which shows good agreement between expected and observed values and coefficients of variation (CV) ranging from 7–18%. In other experiments, repeated analysis of the same human liver sample gave 522 ± 39

fmol/μmol dGuo (N = 7), CV = 7.5%, and repeated analysis of calf thymus DNA gave 1800 ± 47 fmol/μmol dGuo (N = 3), CV = 2.6%. The detection limit for **2** was 0.4 fmol injected on column (signal to noise ratio, 2) and 4.6 fmol/μmol dGuo in DNA, starting with 1 mg DNA.

Levels of adduct **2** measured in 12 human liver samples are summarized in Table 3. It was detected in all samples; mean ± S.D., 534 ± 245 fmol/μmol dGuo. Seven of the liver samples were analyzed without $NaBH_3CN$ treatment during DNA hydrolysis. These samples had considerably lower amounts of adduct **2**, demonstrating that this DNA contained relatively small amounts of preformed adduct **2**. Thus, the measured amount of adduct **2** corresponds mainly to adduct **1**, or a closely related structure, in DNA.

Discussion

The results of this study strongly indicate that N^2-ethylidene-dGuo (**1**) is present in human liver DNA, although we cannot exclude the possibility that a closely related adduct, such as a hydrate, may be converted to adduct **2** by $NaBH_3CN$ reduction. Several lines of evidence support our conclusions. First, clear peaks corresponding to adduct **2** were observed in the LC-ESI-MS/MS chromatograms of all liver samples analyzed by our method, which incorporated $NaBH_3CN$ in the DNA hydrolysis step, thus converting adduct **1** to adduct **2**. Adduct **2** was observed in only small amounts when $NaBH_3CN$ was omitted during DNA hydrolysis, and it was not detected in samples subjected to neutral thermal hydrolysis prior to analysis. These results are completely consistent with the known properties of adducts **1** and **2** (5). Furthermore, our data demonstrate that, although it is possible to form adducts **1** and **2** as artifacts of analysis, this did not occur during our analysis of the human liver samples.

Two previous studies reported the detection of adduct **2** in human white blood cells and urine, respectively, and in both studies the presence of this adduct was discussed with respect to acetaldehyde exposure through alcohol consumption (8,9). It is not clear how adduct **2** would be formed from acetaldehyde, as it is in the wrong oxidation state. Neither of these studies used $NaBH_3CN$ in the analysis, although conversion of adduct **1** to adduct **2** by glutathione or ascorbic acid has been observed in vitro, and could conceivably occur in vivo (7). Our results are the first to demonstrate the presence of adduct **1**, or a closely related structure, in human liver DNA, and to quantify its concentration by conversion to adduct **2**. Quantitation of adduct **2**, as performed here, most likely reflects the same amounts of adduct **1** in DNA, although this is somewhat uncertain because [$^{15}N_5$]**2** was used as internal standard. Employing DNA containing adduct **1** as internal standard is problematic because of its instability.

Although we have no information on alcohol, tobacco use, or other exposures for the subjects who donated the liver samples, it is likely that endogenous sources of acetaldehyde are responsible for at least part of the amounts measured here. Aside from exposures due to ethanol consumption and smoking, acetaldehyde occurs widely in the diet and is present in human blood and breath (1,12,13). Levels of adduct **2** measured in this study, 534 ± 245 fmol/μmol dGuo or about 0.1 adducts per 10^6 nucleotides, are quite consistent with amounts of other endogenous DNA adducts in human tissues, which have been reviewed recently (14). As examples (in adducts per 10^6 nucleotides), levels of M1G from malondialdehyde ranged from 0.06–0.9, etheno adducts from various sources from 0.0006–0.84, and propano-dGuo adducts from aldehydes 0.012–0.40 (14). Levels of adduct **2** reported by Fang and Vaca in white blood cells of alcoholics averaged 0.2 – 0.3 adducts per 10^6 nucleotides; it was not detected in controls.

No data are available on the biological properties of adduct **1** in DNA. Its half-life in DNA of approximately 24 h at 37 °C is probably sufficient for inhibition of DNA synthesis or miscoding events to occur. Several studies have been carried out with adduct **2**. Matsuda et al showed that N^2-ethyl-dGTP was efficiently incorporated opposite dCyd in reactions catalyzed by mammalian DNA polymerases α and δ, and that chain extension occurred rapidly (9). Preferential incorporation of dGMP and dCMP opposite adduct **2** has been observed using the Klenow fragment of *E. Coli* DNA polymerase I (15). Adduct **2** was a strong block to DNA synthesis catalyzed by mammalian DNA pol α, but was efficiently bypassed by DNA pol η, with an accuracy comparable to unadducted dGuo (16). Overall, it appears that adduct **2** has relatively low mutagenic potential in mammalian cells.

Some liver samples contained small amounts of adduct **2** in the absence of $NaBH_3CN$ treatment, similar to previous observations in analyses of white blood cell DNA or urine (8,9). The source of this adduct is not clear. Endogenous reduction of adduct **1** is one possibility, as discussed above. Another is that adduct **2** could be formed as a minor product of DNA ethylation, a process known to occur in humans (17–20).

A limitation of this study is that liver is the only human tissue to which our method has been applied. While the results establish the presence of this adduct in human DNA, liver is not a useful tissue for further studies on the origin of this adduct and its potential role in disease. We do not know whether the method will be sensitive enough to analyze for adduct **1** in more readily available sources of DNA such as white blood cells and exfoliated oral cells, but based on our current detection limit this should be feasible. Our long term goal is to use this assay to explore the relationship between acetaldehyde exposure and susceptibility to cancer in smokers and drinkers.

In summary, our results demonstrate that an acetaldehyde adduct, most likely **1**, is present in human liver DNA, and provide a reliable method for its quantitation as adduct **2**. The most likely source of the adduct is acetaldehyde, a ubiquitous carcinogen and mutagen. The results strongly suggest that this adduct is, at least in part, an endogenous adduct, thus becoming another member of this expanding group of DNA adducts.

Acknowledgments

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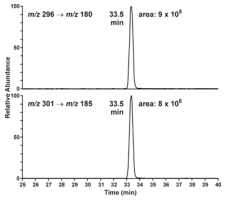

Figure 1.

Chromatograms obtained upon LC-ESI-MS/MS analysis of 10 fmol standard N^2-ethyl-dGuo (**2**) (top) and 100 fmol [$^{15}N_5$]N^2-ethyl-dGuo ([$^{15}N_5$]**2**) (bottom).

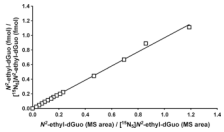

Figure 2.
Calibration curve for N^2-ethyl-dGuo (**2**, 11–555 fmol) and [$^{15}N_5$]N^2-ethyl-dGuo ([$^{15}N_5$]**2**, 500 fmol); $R_2 = 0.99$. Each point is a single determination.

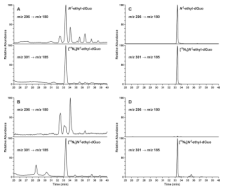

Figure 3.
Chromatograms obtained upon LC-ESI-MS/MS analysis of calf thymus DNA (A, B) and human liver DNA (C, D) by the method described in the text: A and C, $NaBH_3CN$ was used during the DNA hydrolysis step; B and D, $NaBH_3CN$ was not used in the analysis. Areas of the N^2-ethyl-dGuo peak ($\times 10_6$):A, 1.7; B, 0.17; C, 7.5; D, 0.12.

Table 1

Investigation of artifact formation in the analysis of N^2-ethyl-dGuo in DNA.[a]

Human liver sample	Addition of $NaBH_3CN$ during tissue homogenation	Adduct levels (fmol/μmol dGuo)	
		[$^{13}C_2$]2	**2**
A	−	4050	549
	+	31	501
B	−	5550	369
	+	27	360

[a].[$^{13}C_2$]CH_3CHO (28.5 μmol) was added during tissue homogenation and DNA isolation and analysis for [$^{13}C_2$]**2** and **2** were carried out as described in the Experimental Section. $NaBH_3CN$ (1.5 mmol) was added, or not, during tissue homogenation.

Table 2

Analysis of rat liver DNA to which N^2-ethyl-dGuo was added.

N^2-ethyl-dGuo (fmol/mg DNA)[a]		
Added	**Detected[b]**	**CV (%)**
100	109 ± 8	7
200	166 ± 29	18
400	366 ± 33	9
800	734 ± 102	14

a. N^2-ethyl-dGuo in rat liver DNA (194 fmol/mg DNA) was subtracted from each amount detected.

b. Mean ± S.D. (N = 3)

Table 3

Levels of N^2-ethyl-dGuo in human liver DNA.

				N^2-ethyl-dGuo (2) (fmol/μmol dGuo)	
Human liver sample no.	**Amount of DNA analyzed (mg)**	**Sex**	**Age**	**Without $NaBH_3CN$ treatment [a]**	**With $NaBH_3CN$ treatment [a]**
1	0.49	F	51	2	338
2	0.7	M	81	ND[b]	364
3	1.2	F	47	ND	481
4	1.2	M	35	ND	395
5	0.40	M	64	4	343
6	0.19	M	50	ND	257
7	0.38	F	83	2	410
8	1.6	F	51	ND	579
9	0.50	F	46	4	590
10	0.68	M	34	80	690
11	0.50	NA[c]	NA	0	1055
12	0.09	NA	NA	56	908
					534 ± 245

[a] Added in the DNA hydrolysis step

[b] ND, not determined

[c] NA, not available