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Detection of exocyclic 1,N^2-propanodeoxyguanosine adducts as common DNA lesions in rodents and humans

(acrolein/crotonaldehyde)

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ABSTRACT **Exocyclic adducts are unique DNA modifications resulting from binding at two sites of bases that normally are involved in hydrogen-bonding for maintaining the double-helical structure of DNA. These adducts have been shown to be formed in rodents upon exposure to carcinogens. Using a sensitive ^{32}P-postlabeling method combined with high performance liquid chromatography, we obtained evidence that 1,N^2-propanodeoxyguanosine adducts of acrolein (AdG) and crotonaldehyde (CdG) are present in the liver DNA of humans and rodents without carcinogen treatment. The identities of these adducts were verified by cochromatography with the synthetic adduct standards. Further proof of identities was obtained by conversion mediated by nuclease P1 of the labeled AdG and CdG 3′,5′-bisphosphates to their corresponding 5′-monophosphates. This treatment converted the *in vivo* adducts into products that again cochromatographed in a characteristic pattern with the synthetic 5′-monophosphates of AdG and CdG. Using this assay, we also demonstrated the *in vivo* stereoselective formation of one of the AdG isomers. The estimated total levels of modification were 1.0–1.7, 0.2–1.0, and 0.3–2.0 adducts in 10^8 guanine bases in the liver DNA of mice, rats, and humans, respectively. The detection of these adducts in relatively high levels without carcinogen treatment suggests that the endogenous factors such as lipid peroxidation may be important for their formation. This study provides evidence for the presence of acrolein- and crotonaldehyde-derived exocyclic adducts as common lesions in the liver DNA of rodents and humans.**

α,β-Unsaturated aldehydes (enals), such as acrolein and crotonaldehyde, are widespread in the environment. They are generated by burning fats and by cigarette smoking (1) and occur not only as pollutants but also as metabolism products of drugs and carcinogens and by oxidation of membrane lipids (2–4). The endogenous aldehydes may play a role in tissue toxicity (5, 6), as lipid peroxidation has been implicated in carcinogenesis (7–10). The exact mechanism by which lipid peroxidation induces tumors is not clear. While free radicals and malondialdehyde formed during lipid oxidation may be important, enals are also likely to be involved because they are mutagens and carcinogens (11–13). Enals react readily with DNA bases by forming a series of exocyclic propano adducts (14, 15). Immunoassays have demonstrated 1,N^2-propanodeoxyguanosine adducts in the DNA of *Salmonella typhimurium* tester strains and cultured Chinese hamster ovary cells exposed to acrolein or crotonaldehyde (16, 17). Since acrolein and crotonaldehyde are mutagenic in these cell cultures, the presence of 1,N^2-propanodeoxyguanosine adducts in the DNA of these cells suggests that these adducts contribute to mutagenicity. The crotonaldehyde-derived exocyclic adducts are detected in the liver DNA of rats treated with the hepatocarcinogens *N*-nitrosopyrrolidine and crotonaldehyde and in the skin DNA of mice topically treated with crotonaldehyde (18). The acrolein-derived adducts are found in lymphocyte DNA of a dog treated with cyclophosphamide (19). Upon metabolism, *N*-nitrosopyrrolidine and cyclophosphamide yield crotonaldehyde and acrolein, respectively (2, 3). Although the exact role of the propano adducts in carcinogenesis is not fully understood, site-specific mutagenesis studies have shown that transfecting a DNA vector containing a model adduct of 1,N^2-propanodeoxyguanosine into *Escherichia coli* caused either base substitution or frameshift mutations (20, 21).

The ubiquity of enals suggests that 1,N^2-propanodeoxyguanosine adducts of acrolein (AdG) and crotonaldehyde (CdG) may be present in tissue DNA of rodents and humans as common lesions. We have developed a method for detection and quantification of exocyclic adducts in DNA; the method combines HPLC with the ^{32}P-postlabeling technique and enables us to detect as low as 1 adduct in 10^7 to 10^8 bases (22). In this study, we used this method to show that AdG and CdG (Fig. 1) are present in the liver DNA of rodents and humans and that the *in vivo* formation of these adducts appears to be stereoselective.

MATERIALS AND METHODS

DNA Isolation and Hydrolysis and Collection of Adduct Fractions by HPLC. Male A/J mice (25–30 g) and Fischer rats (200–230 g) were purchased from Charles River Breeding Laboratories and housed in polycarbonate cages (25°C ± 2°C, 50 ± 10% relative humidity, and 12-hr light/dark cycle). These animals were fed the modified AIN-76A diet and tap water *ad libitum*. After 3 weeks of acclimatization, all animals were sacrificed. Livers were quickly removed, minced, and frozen at −80°C until DNA isolation. DNA was isolated by a modified Marmur's procedure (23). DNA samples were stored at −80°C until analysis.

DNA (105–300 μg) was obtained from livers of each of four mice and four rats and from each of five humans (two males of ages 37 and 94 years; three females of ages ranging from 50 to 60 years). Human liver DNA was isolated from autopsy samples and was provided by Regina Santella (Columbia University). The DNA was enzymatically hydrolyzed to 3′-monophosphates (22). The enzyme digest was filtered through a 0.2-μm Acrodisc Mini Spike syringe filter (Gelman) and was analyzed by HPLC system 1. Fractions corresponding to AdG 3′-monophosphate and CdG 3′-monophosphate were collected according to the retention times of the synthetic standards (22). To ensure that the assay was free of contamination, blank samples were obtained after injecting 300 μl of water prior to the collection of fractions of DNA hydrolysates from each species. The fractions from the

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Abbreviations: enals, α,β-unsaturated aldehydes; AdG, acrolein-derived 1,N^2-propanodeoxyguanosine; CdG, crotonaldehyde-derived 1,N^2-propanodeoxyguanosine.

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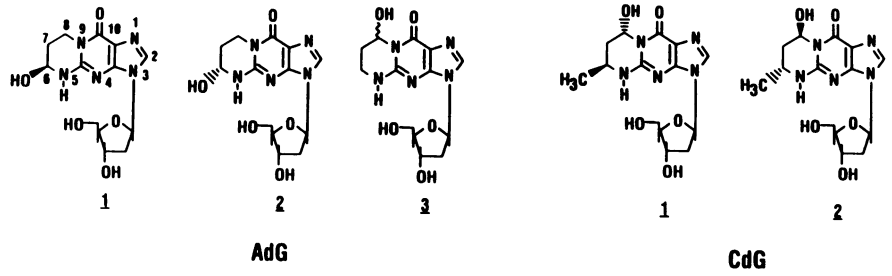

FIG. 1. Structures of AdG and CdG isomers. The absolute configurations of isomers are not determined.

blanks and the DNA hydrolysates were evaporated and reconstituted in 200 μl of water. Two separate experiments were carried out; each used liver DNA of four mice and four rats. However, DNA from the same five human subjects was used in both experiments.

^{32}P-Postlabeling. Thirty microliters of each reconstituted AdG and CdG monophosphate fraction collected as described above was combined, evaporated to dryness in a Speedvac concentrator (Savant), and again reconstituted in 30 μl of distilled water. This sample was then subjected to ^{32}P-postlabeling. Briefly, the aliquot was mixed with 12 μl of the aqueous solution of nuclease P1 (4 μg/μl), 2.5 μl of 1 M sodium acetate (pH 5), and 5.5 μl of 1 mM zinc chloride. Since AdG and CdG 3'-monophosphates are relatively resistant to nuclease P1, this treatment removes the majority of unmodified nucleotides in the collected fractions. The mixture was incubated for 60 min at 37°C, subsequently neutralized with 6 μl Tris base (500 mM), dried in a Speedvac, reconstituted in 10 μl of distilled water, and postlabeled as described (22) to convert the unlabeled monophosphate to a labeled 3',5'-bisphosphate by treatment with [γ-^{32}P]ATP and T4 polynucleotide kinase.

Detection of Adducts. The adduct spots identified on TLC plate were excised, extracted, and subsequently reconstituted in 200 μl of water. The entire sample was then spiked with the authentic standards of AdG and CdG bisphosphates as UV markers and purified by HPLC system 2. The radioactive peaks, corresponding to the UV standards of AdG and CdG, were collected. Fractions were dried *in vacuo*, reconstituted in 200 μl of water, and purified separately by HPLC system 3. Fractions containing AdG and CdG were again collected, dried, and reconstituted in 200 μl of H_2O. Aliquots of these samples (100 μl) were combined, mixed with the UV standards of AdG and CdG, and analyzed in HPLC system 4. To estimate recovery, adduct standards were labeled along with the samples and analyzed in an identical manner. Fractions from the blank samples were analyzed in the same manner.

Confirmation of Identities of Adducts. For confirmation of identities, portions of the purified samples were converted to the 5'-monophosphates by nuclease P1. Comigration of the labeled 5'-monophosphate with the 5'-monophosphate UV markers of AdG and CdG was used as a confirmation of identity. The collection from the ion-pair HPLC (system 5) prior to nuclease P1 treatment was necessary because the 3'-dephosphorylation was retarded in phosphate medium. Aliquots (40 μl) from each fraction were treated with 10 μl of the aqueous solution of nuclease P1 (4 μg/μl), 4 μl of 1 M sodium acetate (pH 5), and 6 μl of 1 mM zinc chloride, and the mixture was incubated at 37°C for 24 or 48 hr for AdG or CdG adducts. After incubation, the mixture was analyzed for AdG and CdG adducts by using HPLC systems 6 and 7 with 3',5'-bisphosphate and 5'-monophosphate UV standards.

Quantitation of Adduct Levels. Recoveries from labeling to final analysis in each assay were determined by the use of adduct standards. Radioactivities associated with the comigrating peaks from DNA of each species were corrected for decay and recovery and were adjusted for dilution in various steps. The levels of each adduct were expressed as μmol of adduct per mol of guanine (based on an [γ-^{32}P]ATP specific activity of 4500 Ci/mmol; 1 Ci = 37 GBq). Since the adduct standards were labeled without collection from HPLC (system 1), the recoveries estimated do not include this step.

HPLC Systems. Waters 501 and/or 510 pumps connected to an automatic gradient controller and a photodiode array detector (Waters, model 990) were used with HPLC systems 1, 2, 3, and 5. HPLC systems 4, 6, and 7 used a UV detector (Waters model 440) and Flow-One β (Radiomatic Instru-

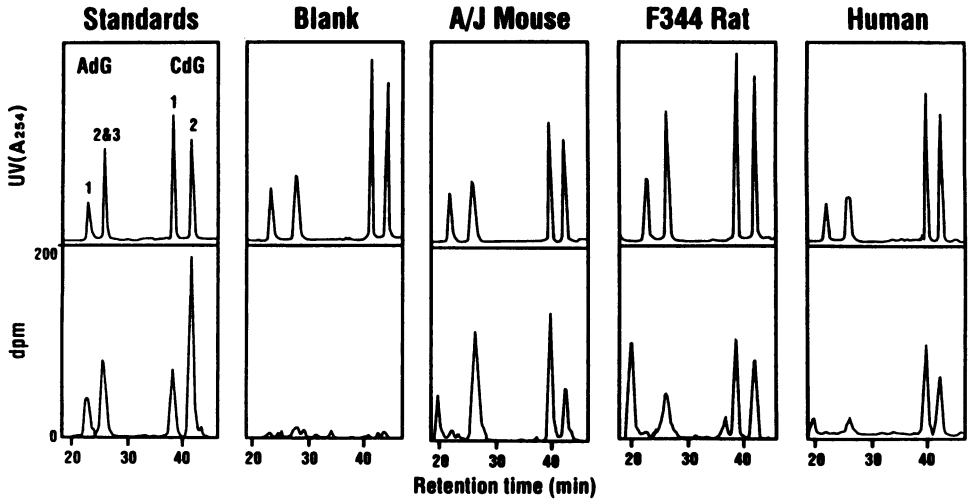

FIG. 2. Comigration of UV markers of the 3',5'-bisphosphates of AdG and CdG with the purified radioactive peaks obtained from liver DNA of various species after ^{32}P-postlabeling. The radioactive peak at 20 min comes from residual 3',5'-bisphosphate deoxyadenosine in AdG fractions collected from DNA hydrolysate. Only a representative chromatogram from each species is shown. HPLC system 4 was used.

Table 1. Retention time (min) of AdG and CdG adducts in HPLC systems

HPLC*	AdG 1	AdG 2 and 3	CdG 1	CdG 2
System 3 (SAX)	32.5	35.8	35.7†	35.7†
System 5 (ion-pair)	45.0	54.0	43.5	48.0

*Systems other than HPLC system 4 (Fig. 2) used to show comigration. SAX, strong anion exchange.
†In system 3, CdG 1 and 2 were eluted as a single peak.

ments and Chemicals, Tampa, FL). For HPLC systems 1, 2, 4, and 5, two Burdick & Jackson (Baxter Health Care, McGaw Park, IL) 5-μm, 4.6 × 250 mm C_{18} reverse-phase columns in series were used at a flow rate of 0.6 ml/min. A linear increase was used for all gradient programs with solvents A and B.

System 1. A: 5 mM Tris HCl (pH 5.8); B: 50% CH_3OH in H_2O; from 0 to 45% B in 60 min.

System 2. A: 50 mM NaH_2PO_4 (pH 5.8); B: 50% CH_3OH in H_2O; isocratic system with 0% B from 0 to 25 min, then from 0 to 20% B in 35 min.

System 3. Two Whatman Partisil 10 strong anion-exchange columns (Baxter) in series; A: NaH_2PO_4 (pH 4.5); B: 50% CH_3OH in H_2O; 10% B isocratic at 0.75 ml/min.

System 4. A: 50 mM NaH_2PO_4 (pH 5.8); B: 50% CH_3OH in H_2O; from 0 to 25% B in 50 min.

System 5. A: 3% acetonitrile in 50 mM triethylammonium bicarbonate (pH 7.4 adjusted with glacial acetic acid); 100% A isocratic.

System 6. Two Waters 10-μm, 3.9 × 300 mm C_{18} reverse-phase columns (Millipore, Milford, MA) in series; solvents A and B as in system 2; isocratic system with 0% B from 0 to 20 min, then from 0 to 20% B in 40 min at 1 ml/min.

System 7. Identical to system 6, except the gradient was from 0 to 30% B in 60 min.

RESULTS

Comigration of Liver DNA Adducts with the Synthetic Adduct Standards. We had earlier characterized the AdG and CdG isomers at the nucleoside level (14, 24). The AdG isomers were separated by HPLC into three peaks designated AdGs 1, 2, and 3; the CdG isomers resolved into two peaks designated CdGs 1 and 2. AdGs 1 and 2 are diastereomers with the hydroxyl group attached to C-6, and they exist in equilibrium in equal amounts. AdG 3 is a regiostereoisomer of AdGs 1 and 2 with the hydroxyl group positioned at C-8; it actually may be composed of two diastereomers, but these isomers were not separated under the conditions used. CdGs 1 and 2 are diastereomers with the hydroxyl group at C-8. Since the absolute configurations of AdG and CdG isomers have not been determined, the stereochemical assignments are arbitrary in Fig. 1. As bisphosphates, the isomers of AdG and CdG were each separated into two peaks. UV markers of the synthetic 3′,5′-bisphosphates of AdG and CdG are shown in Fig. 2 *Upper*. For AdG, the peaks eluted at 22.3 min and 26 min were AdG 1 and AdGs 2 and 3, respectively. CdGs 1 and 2 were eluted at 38.2 min and 41.7 min. These assignments are based upon conversion of the 3′,5′-bisphosphate adducts to their nucleosides with alkaline phosphatase.

After labeling, the one-dimensional TLC provided an initial purification. The adduct areas excised from the TLC plates were extracted and further purified by sequential HPLC. The first reverse-phase HPLC removed bulk radioactivity, which appears as early peaks. The fractions corresponding to AdG and CdG were collected and purified again on a pair of strong anion-exchange columns. The final chromatograms (Fig. 2) show three major radioactive peaks comigrating with the UV standards of AdGs 2 and 3 and CdGs 1 and 2. The radioactivity coeluting with AdG 1 was considerably less than that with AdGs 2 and 3 in all species. Since AdGs 1 and 2 are in equilibrium, both AdG 1 and AdG 2 had to be present in DNA at much lower levels than AdG 3. No significant radioactivity was detected in the blank samples. The control experiments described in this study rule out chromatography as a source of contamination. However, they do not exclude possible artifacts during isolation of DNA. Two pieces of evidence suggest this is highly unlikely: (*i*) DNA isolated from mouse skin showed no detectable levels of CdG (18); (*ii*) the stereochemistry for the formation of AdG *in vivo* was uniquely different from that observed in DNA modified *in vitro* (14). Comigration of DNA adducts with the UV markers was also demonstrated in two other HPLC systems using either a strong anion-exchange column (system 3) or a reverse-phase column with an ion-pair mobile phase (system 5). The retention times are summarized in Table 1. The comigrations serve as an initial identification of these adducts in liver DNA.

Verification of Adduct Identities by Conversion with Nuclease P1. The unequivocal proof of identities is obtained by

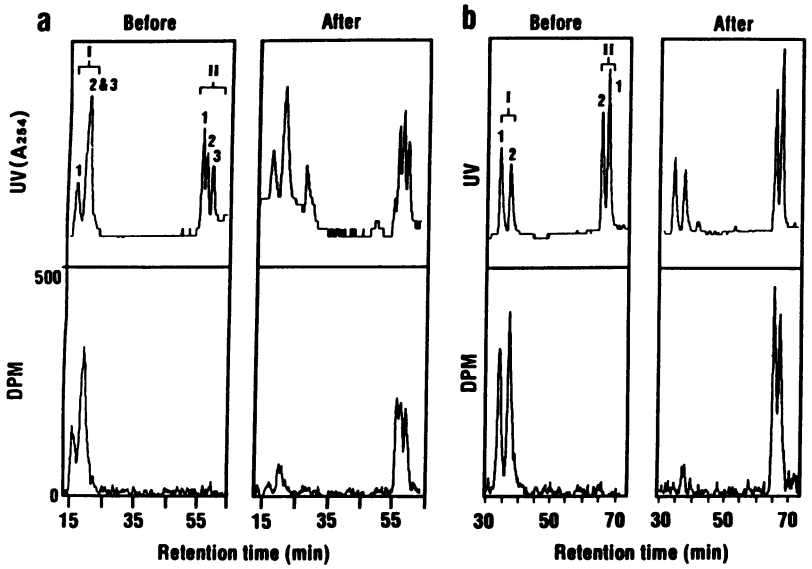

FIG. 3. (*Lower*) Conversion of labeled synthetic 3′,5′-bisphosphates (Before) of AdG (*a*) and CdG (*b*) to the 5′-monophosphates (After) by nuclease P1. (*Upper*) Chromatograms of UV standards of AdG (*a*) and CdG (*b*) in which I indicates 3′,5′-bisphosphates and II indicates 5′-monophosphates.

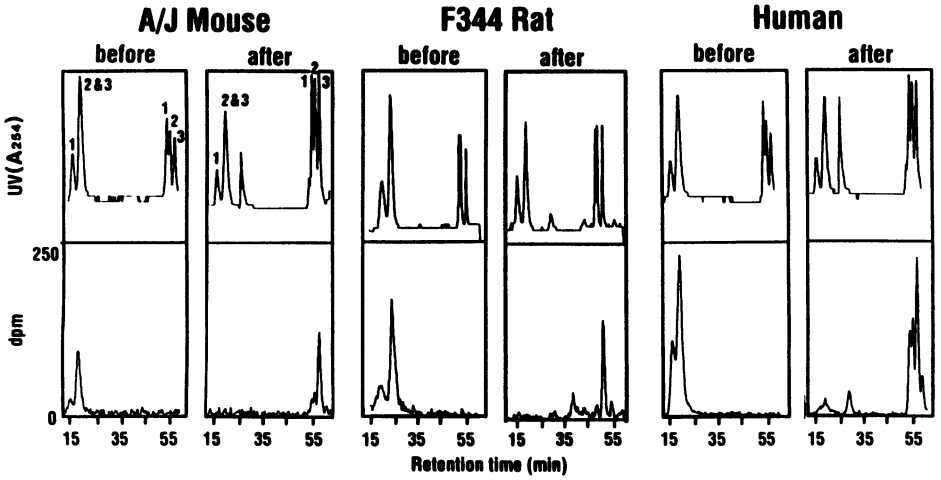

FIG. 4. (*Lower*) Confirmation of identities of AdG adducts in liver DNA of each species by conversion of bisphosphates (Before) to the 5′-monophosphates (After) upon nuclease P1 treatment. (*Upper*) Standards as in Fig. 3. HPLC system 6 was used.

conversion of the 3′,5′-bisphosphates of adducts to the 5′-monophosphates with nuclease P1, an enzyme that selectively hydrolyzes the phosphate at the 3′-position. Fig. 3 depicts the conversion of the ^{32}P-labeled synthetic standards. After conversion, two features are noted in the chromatograms: (*i*) AdG 3 and AdG 2 were coeluted as bisphosphates, but AdG 3 was separated from AdG 2 at the 5′-monophosphate level; and (*ii*) CdG 5′-monophosphates were eluted in a reversed order as their bisphosphates. These features are consistent with those observed with the UV standards (Fig. 3 *Upper*), indicating that nuclease P1 treatment quantitatively converted the AdG and CdG bisphosphates to 5′-monophosphates.

Figs. 4 and 5 show that nuclease P1 treatment converted the 3′,5′-bisphosphates of AdG and CdG detected in liver DNA into products that comigrated with the corresponding 5′-monophosphates. The characteristic pattern of the three peaks of AdG isomers and the opposite order of elution of CdG isomers after the conversion provide further proof of identities of these *in vivo* adducts.

Levels and Stereoselective Formation of AdG and CdG in Liver DNA. The recoveries of adducts in this assay were determined to be 22% for AdG 1, 2, or 3; 8% for CdG 1; and 22% for CdG 2. The low recovery of CdG 1 was due in part to its poor labeling efficiency (22). Together with factors such as dilution and radioactivity decay, these values were used to quantify the levels of adducts in DNA. Although these measurements are relatively crude, the results do show the ranges of adduct levels in liver DNA of various species (Table 2). AdGs 1 and 2 were detected in much lower quantities than AdG 3 and were not consistently detected. AdG 3 was present as the dominant adduct in all species examined. CdG 1 levels were greater than that of CdG 2 in most of the liver DNA analyzed. Among the species studied, humans showed the greatest variability. The total levels of exocyclic adduct modification in the liver DNA of mice are on average 2-fold greater than those found in rats.

DISCUSSION

Results of this study provide unambiguous evidence for the presence of acrolein- and crotonaldehyde-derived 1,N^2-propanodeoxyguanosine adducts in liver DNA of A/J mice, F344 rats, and humans. Since the rodents in this study were not treated with carcinogens it is conceivable that endogenous factors may contribute to adduct formation. One plausible endogenous source is lipid peroxidation, since this normal biochemical process releases a host of enals as end products, including acrolein and crotonaldehyde, although the levels of acrolein and crotonaldehyde detected were low (4). Crotonaldehyde has been found in the blood of healthy individuals (25). Acrolein can also be formed intracellularly by enzymatic oxidation of polyamines (26).

The finding of acrolein- and crotonaldehyde-derived DNA adducts in human liver DNA is intriguing. One of the main

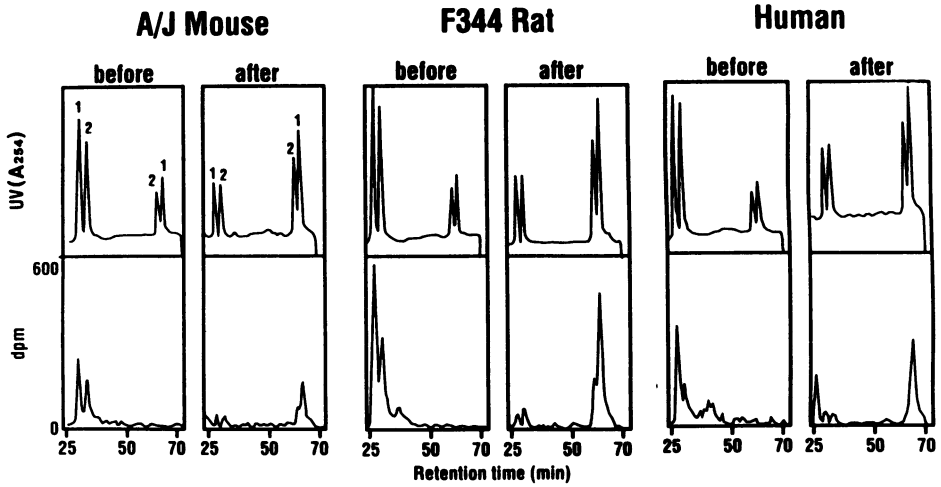

FIG. 5. (*Lower*) Confirmation of identities of CdG adducts in liver DNA of each species by conversion of bisphosphates (Before) to the 5′-monophosphates (After) upon nuclease P1 treatment. (*Upper*) Standards as in Fig. 3. HPLC system 7 was used.

Table 2. Estimated ranges of AdG and CdG adducts detected in human and rodent liver DNA

Species	Adducts, μmol/mol of guanine			
	AdG 3	CdG 1	CdG 2	Total
Mouse	0.23–0.67	0.63–0.86	0.10–0.16	0.96–1.69
Rat	0.01–0.04	0.14–0.74	0.05–0.20	0.20–0.98
Human	0.03–0.74	0.14–1.03	0.13–0.34	0.30–2.11

exogenous exposures to acrolein and crotonaldehyde in humans is by smoking. Unfortunately, we lack information regarding the smoking status of these individuals from whom the liver tissues were obtained. Diets containing low levels of aldehydes, nitrosamines such as *N*-nitrosopyrrolidine, or its precursor amine, which can be endogenously nitrosated, may also contribute to the formation of AdG or CdG *in vivo*. However, the amounts of these compounds in diets are usually quite low. The significant levels of modification of the liver DNA of untreated animals suggest an important endogenous pathway for the formation of these adducts. Randerath *et al.* (27) also detected by the ^{32}P-postlabeling method indigenous adducts (I-compounds) of unknown source and identity in tissue DNA that were not directly related to carcinogen exposure.

In an earlier study of *in vivo* DNA adduction by *N*-nitrosopyrrolidine using the ^{32}P-postlabeling method, we detected a background adduct in rat liver DNA that was chromatographically similar to CdG. However, the identity of this adduct was not rigorously studied (18). In the investigation of vinyl chloride-induced etheno adducts, Fedtke *et al.* (28) observed a low level of N^2,3-ethenoguanine in the liver DNA of untreated rats. More recently, other etheno adducts, 1,N^6-ethenoadenine and 3,N^4-ethenocytosine, also were found in liver of rats (29). In this study, we did not detect 1,N^2-ethenodeoxyguanosine, possibly because of its low level of formation as compared with the propano adducts. The present study demonstrates that the exocyclic propano adducts are present in human DNA and that they appear to be common DNA lesions in the species studied so far. Previously both hemoglobin and urinary alkylated adducts were detected as background in humans (30, 31). Our results also show that among AdG isomers, AdG 3 appears to be preferentially formed *in vivo*. It is possible, however, that AdGs 1 and 2 are initially formed but are less stable or more efficiently repaired than AdG 3. Although the sources responsible for *in vivo* formation of these exocyclic adducts are still uncertain, there is ample chemical evidence supporting the derivation of the propano and etheno adducts from acrolein, crotonaldehyde, and *trans*-4-hydroxy-2-nonenal (14, 32–34). Clearly, more studies are needed to establish the involvement of these pathways *in vivo*.

The potential roles of exocyclic adducts in carcinogenesis are underscored by several observations: they are formed upon carcinogen treatments (35), they are mutagenic lesions (20, 21, 36, 37), and some of them appear to be persistent in target tissues (38). However, *in vitro* repair assays of such lesions have shown rather efficient repair by purified glycosylase from *E. coli* and humans (39, 40). Regardless of the rates of their repair, exocyclic adducts represent a class of recently identified *in vivo* DNA lesions. Other known common endogenous DNA lesions include oxidative damage, deamination, methylation, and apurinic sites. All of these, together with exocyclic adducts, constitute DNA damage that results from normal biochemical and physiological functions of cells. When one considers the high frequency with which these lesions occur in tissue DNA and their potential mutagenic properties, the presence of these lesions in tissue DNA could very well be involved in the development of cancers.

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1. International Agency for Research on Cancer (1979) *IARC Monogr. Eval. Carcinog. Risk Chemicals Man* **19**, 479–495.
2. International Agency for Research on Cancer (1982) in *IARC Monogr. Eval. Carcinog. Risk Chem. Man* **26**, 165–202.
3. Wang, M. Y., Chung, F.-L. & Hecht, S. S. (1988) *Chem. Res. Toxicol.* **1**, 28–31.
4. Esterbauer, H., Zollner, H. & Schaur, R. J. (1990) in *Membrane Lipid Oxidation*, ed. Vigo-Pelfrey, C. (CRC, Boca Raton, FL), Vol. 1, pp. 239–283.
5. Comporti, M. (1985) *Lab. Invest.* **53**, 599–623.
6. Schaur, R. J., Zollner, H. & Esterbauer, H. (1991) in *Membrane Lipid Oxidation, Vol. II*, ed. Vigo-Pelfrey, C. (CRC, Boca Raton, FL), Vol. III, pp. 141–163.
7. Joel, S. K., Lalwani, N. D. & Reddy, J. K. (1986) *Cancer Res.* **46**, 1324–1330.
8. Rushmore, T. H., Lim, Y. P., Farber, E. & Ghohal, A. K. (1984) *Cancer Lett.* **24**, 251–255.
9. Shamberger, R. J. (1972) *J. Natl. Cancer Inst.* **48**, 1491–1497.
10. Bartsch, H., Hietanen, E. & Malaveille, C. (1989) *Free Radical Biol. Med.* **7**, 637–644.
11. Marnett, L. J., Hurd, H. K., Hollstein, M. C., Levin, D. E., Esterbauer, H. & Ames, B. N. (1985) *Mutat. Res.* **148**, 25–34.
12. Chung, F.-L., Tanaka, T. & Hecht, S. S. (1986) *Cancer Res.* **46**, 1285–1289.
13. Cohen, S. M., Garland, E. M., St. John, M., Okamura, T. & Smith, R. A. (1992) *Cancer Res.* **52**, 3577–3581.
14. Chung, F.-L., Young, R. & Hecht, S. S. (1984) *Cancer Res.* **44**, 990–995.
15. Smith, R. A., Williamson, D. S., Cerny, R. L. & Cohen, S. M. (1990) *Cancer Res.* **50**, 3005–3012.
16. Foiles, P. G., Akerkar, S. A. & Chung, F.-L. (1989) *Carcinogenesis (London)* **10**, 87–90.
17. Foiles, P. G., Akerkar, S. A., Miglietta, L. M. & Chung, F.-L. (1990) *Carcinogenesis (London)* **11**, 2059–2061.
18. Chung, F.-L., Young, R. & Hecht, S. S. (1989) *Carcinogenesis (London)* **10**, 1291–1297.
19. Wilson, V. L., Foiles, P. G., Chung, F.-L., Povey, A. C., Frank, A. A. & Harris, C. C. (1991) *Carcinogenesis (London)* **12**, 1483–1490.
20. Moriya, M., Marinelli, E., Shibutani, S. & Joseph, J. (1989) *Proc. Am. Assoc. Cancer Res.* **30**, 555 (abstr.).
21. Benamira, M., Singh, U. & Marnett, L. J. (1992) *J. Biol. Chem.* **267**, 22392–22400.
22. Nath, R. G., Chen, H.-J. C., Nishikawa, A., Young-Sciame, R. & Chung, F.-L. (1994) *Carcinogenesis (London)* **15**, 979–984.
23. Marmur, J. (1961) *J. Mol. Biol.* **3**, 208–218.
24. Chung, F.-L. & Hecht, S. S. (1983) *Cancer Res.* **43**, 1230–1235.
25. Zlatkis, A., Poole, C. F., Brazeli, R., Bafus, D. A. & Spencer, P. S. (1980) *J. Chromatogr.* **182**, 137–145.
26. Russo, A., Mitchell, J. B., DeGraff, W., Friedman, N. & Gamson, J. (1985) *Cancer Res.* **45**, 4910–4914.
27. Randerath, K., Li, D., Nath, R. & Randerath, E. (1992) *Exp. Gerontol.* **27**, 533–549.
28. Fedtke, N., Boucheron, J. A., Walker, V. E. & Swenberg, J. A. (1990) *Carcinogenesis (London)* **11**, 1287–1292.
29. Barbin, A., Ghissassi, E., Nair, J. & Bartsch, H. (1993) *Proc. Am. Assoc. Cancer Res.* **34**, 136.
30. Farmer, P. B., Bailey, E., Naylor, S., Anderson, D., Brooks, A., Cushnir, J., Lamb, J. H., Sepai, O. & Tang, Y. S. (1993) *Environ. Health Perspect.* **99**, 19–24.
31. Shuker, D. E., Prevost, V., Friesen, M. D., Lin, D., Ohshima, H. & Bartsch, H. (1993) *Environ. Health Perspect.* **99**, 33–37.
32. Sodum, R. S. & Chung, F.-L. (1988) *Cancer Res.* **48**, 320–323.
33. Sodum, R. S. & Chung, F.-L. (1991) *Cancer Res.* **51**, 137–143.
34. Chen, H.-J. C. & Chung, F.-L. (1994) *Proc. Am. Assoc. Cancer Res.* **35**, 155 (abstr.).
35. Singer, B. & Bartsch, H. (1986) *IARC Sci. Publ.* **70**, 3–14.
36. Cheng, K. C., Preston, B. D., Cahill, D. S., Dosanjh, M. K., Singer, B. & Loeb, L. A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 9974–9978.
37. Palejwala, V. A., Simha, D. & Humayun, M. Z. (1991) *Biochemistry* **30**, 8736–8743.
38. Swenberg, J. A., Fedtke, N., Ciroussel, F., Barbin, A. & Bartsch, H. (1992) *Carcinogenesis (London)* **13**, 727–729.
39. Matijasevic, Z., Sekiguchi, M. & Ludlum, D. B. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 9331–9334.
40. Singer, B., Antoccia, A., Basu, A. K., Dosanjh, M. K., Fraenkel-Conrat, H., Gallagher, P. E., Kusmierek, J. T., Qiu, Z. H. & Rydberg, B. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 9386–9390.