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ORIGINAL RESEARCH

Quantitation of normal and formaldehyde-modified deoxynucleosides by high-performance liquid chromatography/UV detection

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ABSTRACT: A sensitive and selective method was developed for the first time to quantify simultaneously the normal and formaldehyde (FA)-modified bases in human placental DNA treated with 100 ppm FA for 20 h at 37°C. Digestion of DNA to deoxynucleosides with DNase I, phosphodiesterase and alkaline phosphatase occurred in that order with centrifugation steps. The normal and FA-modified deoxynucleosides were then resolved from one another and reagent blank interferences to produce selective separation through high performance liquid chromatography–ultraviolet detection at 254 nm. A C_{18} reversed-phase column facilitated the resolution using 5 mM ammonium acetate and a gradient of 0–6% methanol at flow rates of 0.3–1.4 mL/min before column cleaning. The lower quantifiable limits for deoxyadenosine, deoxyguanosine, deoxycytidine, thymidine, N^6-hydroxymethyldeoxyadenosine (N^6-dA), N^2-hydroxymethyldeoxyguanosine (N^2-dG) and N^4-hydroxymethyldeoxycytidine (N^4-dC) were 11, 7.6, 12, 15, 10, 10 and 22 pmol, respectively. The abundance order of the modified deoxynucleosides was N^6-dA > N^2-dG > N^4-dC. dT did not form hydroxymethyl derivatives. The respective concentrations were about 6.0, 10.0 and 23 pmol of modified deoxynucleosides in 80 μg of human placental DNA after treatment with 100 μg/mL of formalin for 20 h at 37°C. The stabilities of N^6-dA and N^2-dG were much better at –20°C than at 25°C, where the respective halftimes were about 50.1 and 21.0 h.

KEYWORDS: DNA adducts; formaldehyde; hydroxymethyldeoxynucleosides; HPLC/UV; biomarkers

INTRODUCTION

Formaldehyde (FA) is widely used in hospitals and industry (WHO, 1989; US Department of Health and Human Services, 1999). Several studies indicate that FA causes nasal squamous cell carcinomas in rats (Swenberg *et al.*, 1980, 1983; Kerns *et al.*, 1983). Two Danish studies found relative risks of 3.0 for nasopharyngeal cancer in male workers exposed to ≥1 ppm FA for at least 10 years (Olsen and Asnaes, 1986; Hansen and Olson, 1995). Vaughan *et al.* (2000) also reported that occupational exposure to FA in the USA increased the risk for nasopharyngeal cancer at five cancer registries (odds ratio = 3.0 for people exposed to >1.1 ppm for over 10 years). IARC (1995) considers FA to be a probable human nasal carcinogen (group A2). The biological effects of FA arise from its interaction with proteins and nucleic acids (Morgan, 1997).

Biological monitoring of exposure to FA is more directly related to adverse health effects than any environmental measurement. No significant differences were found in pre- and post-exposure blood FA concentrations and urine formic acid for low FA air concentrations (Gottschling *et al.*, 1984; Marquardt *et al.*, 1999). The amount of DNA–protein crosslinking (DPC) has been proposed as a potential biomarker for exposure and genotoxic damage caused by FA inhalation (Shaham *et al.*, 2003). However, DPC is not a unique biomarker for FA, and occurs after exposure to compounds other than FA, such as acetaldehyde, Cr(VI) and hydroxyl radical (Feron *et al.*, 1991). DNA adducts in the target organ may be potential dosimeters and also predictive markers of effect for carcinogens (Fennell, 1994). ^{32}P-postlabeling can distinguish DNA adducts of high molecular weight compounds like aromatics from non-modified DNA, but not those of low molecular weight agents like FA (Que Hee, 1993). A major alternative is to detect the free bases, deoxynucleosides and deoxynucleotides that can be produced from modified DNA by conventional enzymic hydrolysis.

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Abbreviations used: dA, deoxyadenosine; dC, deoxycytidine; dG, deoxyguanosine; DPC, DNA–protein crosslinking; dT, thymidine; FA, formaldehyde; LQL, lower quantifiable limit.

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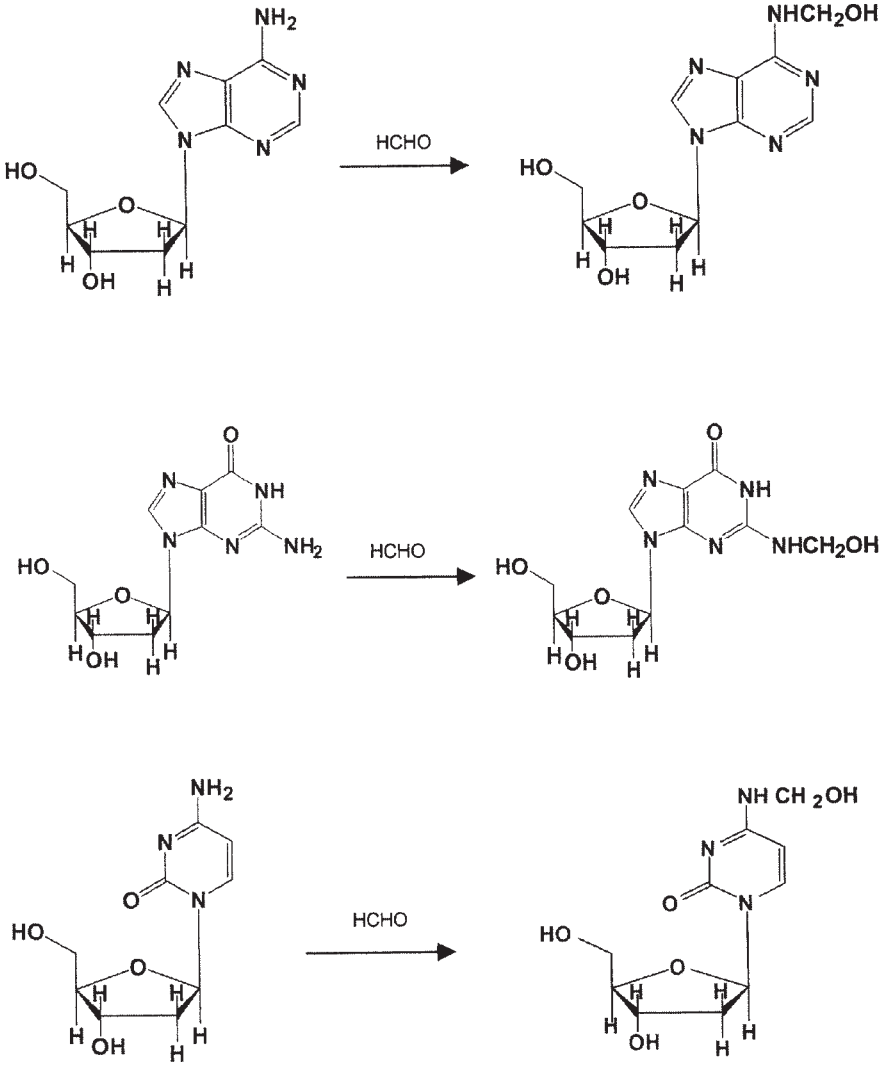

Figure 1. FA reacts with deoxynucleosides (top left, deoxyadenosine; middle left, deoxyguanosine; bottom left, deoxycytidine) to form hydroxymethyl deoxynucleosides.

FA reacts with DNA to form hydroxymethyl derivatives (Fig. 1; Feldman, 1973; Beland *et al.*, 1984; Casanova *et al.*, 1989; Fennell, 1994). Beland *et al.* developed a high performance liquid chromatography (HPLC) method which allowed detection of FA-modified deoxynucleosides from the DNA of Chinese hamster ovary cells at an ultraviolet (UV) wavelength of 254 nm. This method was qualitative only, since resolution of peaks from DNA samples was not achieved. Since DNA adducts are more selective than DPC, there was still need for a sensitive, accurate biomarker for FA exposure.

The present paper describes a new highly sensitive, accurate and selective reversed-phase HPLC/UV detection method which resolves modified deoxynucleosides from their non-modified deoxynucleosides and reagent/DNA interferences, allowing accurate quantitation for the first time. The stabilities of two hydroxymethyldeoxynucleosides were determined for the first time also in ruggedness studies.

EXPERIMENTAL

Chemicals. Formalin (37%, w/w, FA in 10% methanolic aqueous solution) was purchased from Aldrich (Milwaukee, WI, USA). Deoxyadenosine (dA), deoxyguanosine (dG), deoxycytidine (dC), thymidine (dT), human placental DNA (type XIII), and alkaline phosphatase (type XVII from human placenta) were purchased from Sigma (St Louis, MO, USA). DNase I (from bovine pancreas) and phosphodiesterase I (from *Crotalus adamanteus*) were obtained from ICN (Costa Mesa, CA, USA). Bis–Tris buffer, disodium EDTA, methanol (Optima), and ammonium acetate (HPLC grade) were purchased from Fisher Scientific (Tustin, CA, USA).

Apparatus. The HPLC system was a model 1090 liquid chromatograph from Hewlett-Packard (HP) (Palo Alto, CA, USA) with a HP 1050 UV diode array variable wavelength detector, and an HP 3396 series II integrating recorder. A 10 μL manual injection loop was loaded with 100 μL. The C_{18} reversed-phase analytical column (Ultrasphere Ods 5 μm, 250 mm × 4.6 mm) was from Beckman (Fullerton, CA, USA). A Beckman L8-70M ultracentrifuge and Beckman centrifuge tubes (VWR, San Diego, CA, USA) were used in the enzymatic hydrolysis procedure for sample preparation.

Preparation of hydroxymethyldeoxynucleosides and their standard curves. No commercial hydroxymethyldeoxynucleoside standards are available. Standards were synthesized (Gehrke, 1984) by reacting deoxynucleosides with FA in 5 mM bis–Tris buffer–0.1 mM disodium EDTA buffer. Each deoxynucleoside and the mixture (0, 0.5, 1.0, 2.0 and 4.0 nmol/mL) were exposed to 100 ppm FA (1.0 μL 10% formalin) at 37°C for 6 h. The deoxynucleosides were resolved by HPLC at 254 nm with a C_{18} reversed-phase analytical column by gradient elution with pH 6.0 5 mM ammonium acetate at 0.3 mL/min for 0–20 min and a gradient of 0–6% methanol and a flow rate of 0.3–1.4 mL/min between 20 and 30 min, followed by isocratic conditions of 6% methanol/5 mM ammonium acetate at 1.4 mL/min between 30 and 50 min. Quantitation of normal deoxynucleosides was performed by the method of external standards.

The standard curves for each hydroxymethyldeoxynucleoside were obtained by the following procedure. After FA reaction with each pure deoxynucleoside, the normal deoxynucleoside was quantified by external standards. The difference between the moles of the original and the unreacted deoxynucleoside at each specific concentration of original deoxynucleoside was the moles of hydroxymethyldeoxynucleoside formed. Plots of average peak area of the modified deoxynucleoside corrected for any blank interference vs moles injected of modified deoxynucleoside were then subjected to linear regression analysis to obtain slopes, intercepts, standard deviations of slopes and intercepts, correlation coefficients and *p*-values. These standard curves were then utilized for deoxynucleoside mixture and hydrolysate determinations. All concentrations were evaluated at least in triplicate.

The above indirect method was verified by repetitively collecting pure N^6-dA and N^2-dG directly from the HPLC column using a methanol/water gradient of 5–10% over 20 min and 2–8% over 20 min at a flow rate of 2 mL/min, respectively. The eluates were collected at –10°C with storage at –20°C. The excess water and methanol were evaporated at 40°C until constant weight. The normal deoxynucleoside was quantified directly to obtain the purity of the collected solid. Standard curves were then constructed using ammonium acetate buffer as above to compare response factors (slopes).

Stability of hydroxymethyldeoxynucleosides. N^6-hydroxymethyldeoxyadenosine (N^6-dA) and N^2-hydroxymethyldeoxyguanosine (N^2-dG) were freshly synthesized by the method specified above. Fractions were then collected from the HPLC column by a tube at –10°C. Each sample was injected into the HPLC/UV immediately to define the initial N^6-dA and N^2-dG concentrations by quantifying unmodified deoxynucleoside relative to the initial unreacted concentration. The uninjected portions were aliquoted into 2 ml vials and stored at room temperature (24°C) and –20°C for the stability experiments. The stored samples were analyzed every day for 2 weeks, and then weekly for up to 6 months. Prior to analysis, the samples stored at –20°C were thawed to ambient temperature. For each degradation curve, at least six points were used in a time frame corresponding to one to three half-lives or until equilibrium occurred.

Reaction of human placental DNA with FA, and enzymatic hydrolysis of DNA. The optimized hydrolysis procedure is presented below: a 1.0 mL solution containing 80 μg human placental DNA and 100 ppm FA (1.0 μL of 10% formalin) in pH 4.5 40 mM sodium acetate buffer was incubated for 20 h at 37°C. The mixture was cooled on ice, and the DNA precipitated by addition of 30 μL of 1 M sodium chloride and then 800 μL ice-cold ethanol. The DNA was recovered by centrifugation (14 000 ***g*** for 10 min) and dissolved in 1.0 ml of 5 mM bis–Tris–0.1 mM disodium EDTA buffer, pH 7.1. The reagent blank sample had 10% methanolic water added instead of 37% FA (formalin contains 10% methanol). The DNA solutions were hydrolyzed by incubation with DNase I (10 U) in 10 mM magnesium chloride at 37°C for 30 min. This was followed by addition of human placental alkaline phosphatase (0.1 U) and snake venom phosphodiesterase (0.1 U) at 37°C for 30 min to liberate the deoxynucleosides. The solution was centrifuged at 20 000 ***g*** for 10 min to remove precipitated material. Aliquots of the supernatant were injected into the HPLC. The same gradient elution program was used as for the deoxynucleosides above. However, peaks related to the enzyme blank continued to elute after 50 min. Column cleaning with 25% methanol at 1.5 mL/min for 15 min and then 5 mM ammonium acetate at 0.3 mL/min for 10 min was then necessary to prevent the appearance of 'ghost' peaks in subsequent runs. Triplicate samples of DNA and reagent blanks were processed and analyzed. The supernatants were stored at –20°C.

Statistical analyses. Average area versus moles injected plots were generated for each treatment from the appropriate triplicates data. The linear portion of each curve was fitted with a least squares regression line using EXCEL. The slopes and their standard deviations were compared by analysis of variance (ANOVA) and the Student *t*-test. Correlation coefficients (*r*) and *p*-values were also calculated for all linear regression equations. *P*-Values of ≤0.05 were considered significant. Detection limits (DL) were defined as three times the noise and lower quantifiable limits (LQL) were defined as 10 times the noise.

RESULTS

All deoxynucleosides and modified deoxynucleosides eluted with baseline resolution resolved from enzyme blank interferences for the optimized procedure (Fig. 2). Each hydroxymethyldeoxynucleoside eluted soon after dA, dG and dC, but dT did not react. The modified deoxynucleoside eluted slower than its

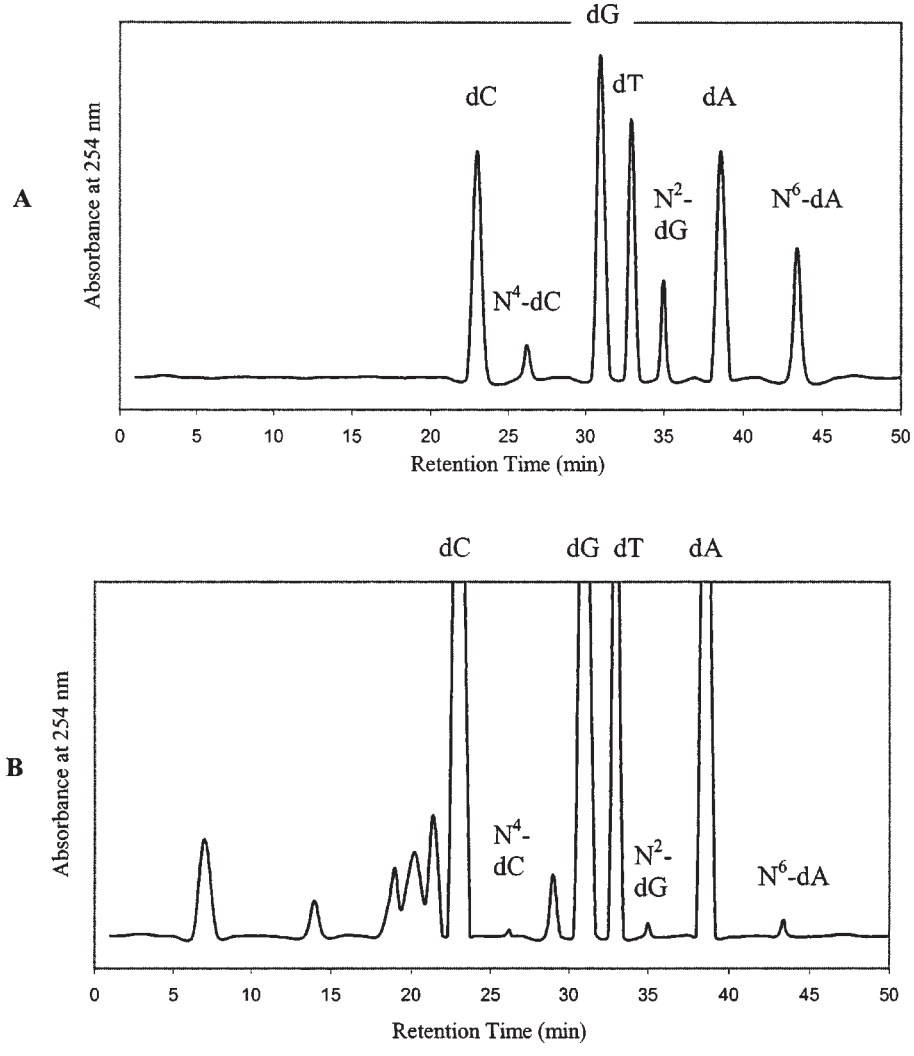

Figure 2. Chromatograms of the reverse-phase C_{18} column HPLC separation of normal deoxynucleosides, modified deoxynucleosides, and enzyme interferences. (A) deoxynucleosides + 100 ppm FA (6 h, 37°C); (B) 80 μg human placental DNA + 100 ppm FA (20 h, 37°C). The deoxynucleosides were resolved by HPLC at 254 nm by gradient elution with pH 6.0 5 mM ammonium acetate at 0.3 mL/min for 0–20 min and a gradient of 0–6% methanol and a flow rate of 0.3–1.4 mL/min between 20 and 30 min, and then 6% methanol from 30 to 50 min at 1.4 mL/min.

normal deoxynucleoside because the latter is more polar. Only one extra peak resulted from the reaction of FA with each pure deoxynucleoside.

HPLC program optimization

To optimize the separation on this column (Fig. 2), the effects of ammonium acetate concentration, flow rate and methanol concentration on the elution of deoxynucleosides generated from DNA and enzyme reagent blank peaks were investigated starting with the conditions of Beland *et al.* (1984). Those conditions involved gradient elution with 10 mM ammonium acetate and up to 20% methanol at 2 mL/min. The three parameters were changed over the first 20 min to move dC to a new elution time of 23 min which no longer interfered with the last enzyme peak at 21 min. However, retention times of subsequent peaks were then too long. This problem was overcome by increasing flow rate from 0.3 to 1.4 mL/min and percentage methanol from 0 to 6% at 20–30 min, and then maintaining 6% methanol at 1.4 mL/min to 50 min. When methanol concentration was higher than 6%, the noise peaks from bubble formation became too high for sensitive determination. In addition, an enzyme blank peak interfered with dG at above 15% methanol. DNA digests also caused enzyme-related peaks to elute after 50 min, necessitating the cleaning of the column before the next injection to prevent elution of 'ghost' peaks in subsequent runs.

Table 1. Linear regression parameters for normal and modified deoxynucleosides. The ± quantities are standard deviations for $n = 4$

	Slope ($\times 10^{15}$), area/mol cm	SDA,[a] pmol/10 µL	r^2	DL,[b] pmol/10 µL	LQL,[c] pmol/10 µL
dA	3.03 ± 0.20	1.1	0.996[d]	3	11
dG	3.16 ± 0.26	0.75	0.993[d]	2	7.6
dC	1.69 ± 0.13	1.2	0.994[d]	4	12
dT	1.88 ± 0.15	1.5	0.994[d]	5	15
N^6-dA	3.55 ± 0.29	1.0	0.987[d]	3	10
N^2-dG	3.88 ± 0.39	1.0	0.986[d]	3	10
N^2-dC	1.73 ± 0.46	2.2	0.954[d]	6	22

[a] SDA, standard deviation of analytical results (Skoog *et al.*, 1998).
[b] DL, three times SDA.
[c] LQL, 10 times SDA.
[d] $p \leq 0.05$.

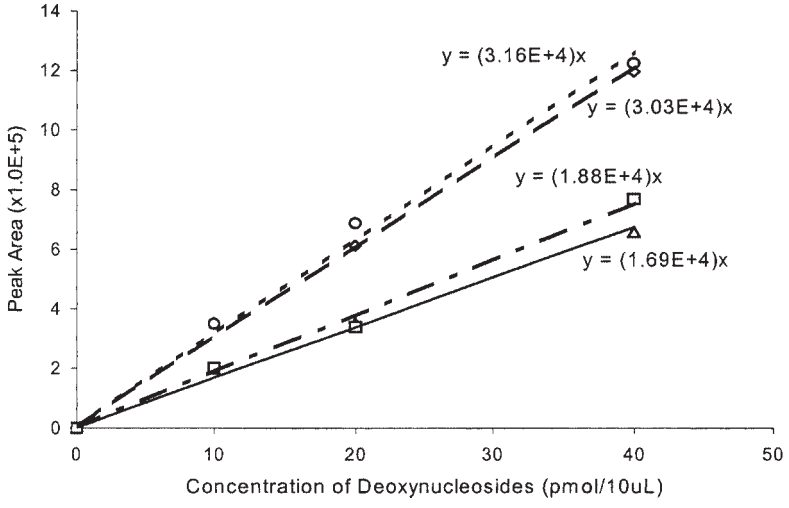

Figure 3. Calibration curves and regression equations describing the relationship between its peak area and the amount of deoxynucleoside (dA, ◇; dG, ○; dC, △; dT, □). The slope units are in area units/pmol in 10 µL injected.

Standard curves

The LQLs for dA, dG and dT are 7.6–15 pmol/10 µL (Table 1). The LQL for N^6-dA and N^2-dG is 10 pmol/10 µL, but it is 22 pmol/10 µL for N^4-dC. Figure 3 illustrates the linearity of the standard curve of the normal deoxynucleosides. The slopes for dA and dG are equivalent ($p > 0.05$), as are those for dC and dT. Standard curves for the modified deoxynucleosides obtained by the indirect method are shown in Figure 4. The slopes for N^6-dA and N^2-dG show no significant difference, but both slopes are significantly bigger than the slope for N^4-dC at $p \leq 0.05$. The slopes of each normal deoxynucleoside and its modified deoxynucleoside are also not significantly different ($p > 0.05$). For all normal and modified deoxynucleoside curves, $r^2 > 0.95$ were observed, with the curve for N^4-dC having the lowest r^2, but still having $p \leq 0.05$.

The initial purities of N^6-dA and N^2-dG collected directly from the HPLC column using the methanol–water gradient were 97.3 ± 1.4 and 94.2 ± 2.1%, respectively. The respective slopes analyzed under the same chromatographic conditions were (3.06 ± 0.43) × 10^{15} area units/(mol cm) and (3.53 ± 0.08) × 10^{15} area units/(mol cm). These slopes were not significantly different ($p > 0.05$) from those obtained by the indirect method. Therefore, it is inferred that the indirect quantitation method for N^4-dC will be accurate.

The thermal stabilities of N^6-dA and N^2-dG are provided at −20 and 25°C in Figure 5. After 14 days at 25°C, only 14.2% remained for N^6-dA and 7.4% for N^2-dG with respective half-lives of 50.1 ± 4.9 and 21.0 ± 1.1 h, assuming pseudo-first-order kinetics. At –20°C, both were stable (>95%) for at least 10 days. After one month, 83.3% remained for N^6-dA and 77.1% for N^2-dG. After 6 months, 32.9 and 20.2% remained. The

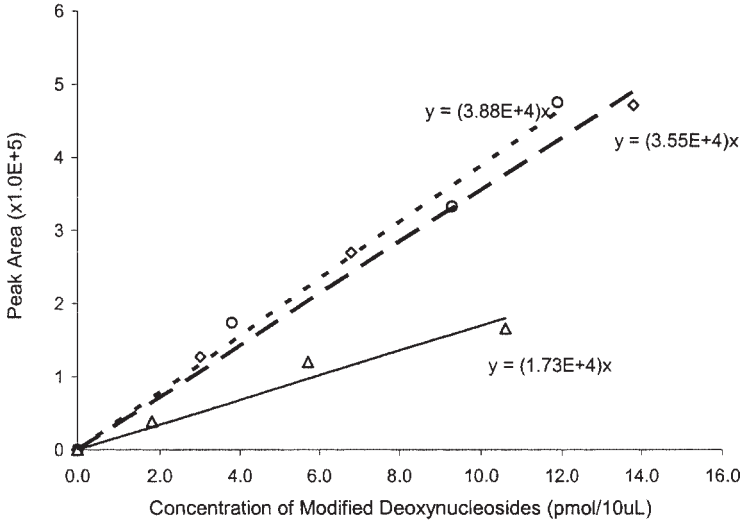

Figure 4. Calibration curves and regression equations describing the relationship between its peak area and the amount of modified deoxynucleoside (N^6-dA, ◇; N^2-dG, ○; N^4-dC, △). The slope units are in area units/pmol in 10 μL injected.

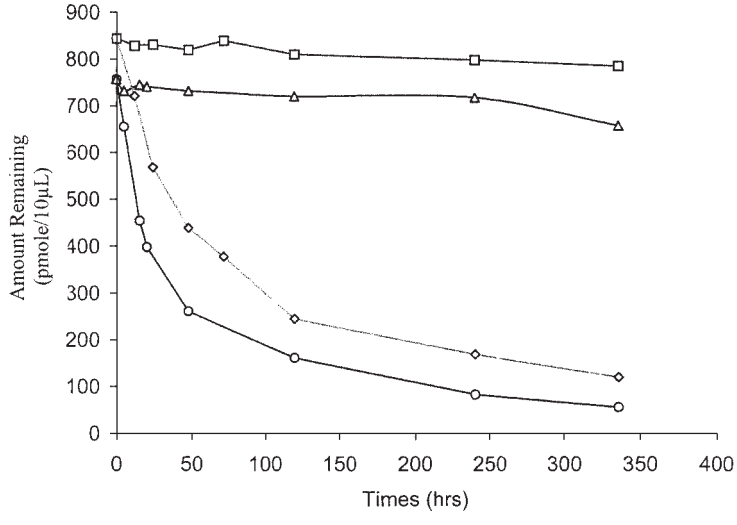

Figure 5. Influence of storage temperature on the degradation of N^6-dA and N^2-dG (N^6-dA at 25°C, ◇; N^6-dA at –20°C, □; N^2-dG at 25°C, ○; N^2-dG at –20°C, △). The size of the symbol is about the size of the standard deviations.

respective half-lives were 112 ± 8 and 78 ± 7 days, assuming first-order decay kinetics.

Separation of normal and modified deoxynucleosides from DNA

A typical chromatogram of human placental DNA after incubation with FA is shown in Fig. 2(B) at the optimized chromatographic conditions. Four normal deoxynucleosides and three modified deoxynucleosides showed baseline separation. The retention times of dC, N^4-dC, dG, dT, N^2-dG, dA and N^6-dA were 23.0, 26.2, 31.0, 33.0, 35.0 38.6 and 43.4 min, respectively. The CVs for retention times were less than 1%. Resolution of enzyme blank impurities and dC, and dC and N^4-dC, was therefore successfully accomplished to allow accurate quantitation.

Analysis of DNA for normal and modified deoxynucleosides

The mol % deoxynucleosides for dA, dT, dG, and dC in human placental DNA were 28.8 ± 2.9, 27.0 ± 2.3, 22.9 ± 1.8 and 21.2 ± 2.0%, respectively. The (G + C)

Table 2. Amount (pmol) of modified deoxynucleosides in 80 μg human placental DNA incubated with 100 ppm FA[a]

	N^4-dC	N^2-dG	N^6-dA
DNA	<DL[b]	<DL[b]	<DL[b]
DNA	6.03	10.04	23.1
+FA	±0.80[c]	±0.91[c]	±1.8[c]

[a] 10 μL injection.
[b] Detection limit.
[c] Standard deviation (n = 3).

content of human placental DNA is thus 44.2 ± 3.8%. Table 2 shows the amount (pmol) of modified deoxynucleosides in 80 μg human placental DNA after 20 h incubation with 100 ppm FA at 37°C. FA reacts with the amino group of the adenine moiety more easily than that of the guanine moiety, the latter being as reactive as the amino group of the cytosine ring.

DISCUSSION

The previously published HPLC/UV method (Beland *et al.*, 1984) did not allow quantitative analysis of FA-modified deoxynucleosides because of resolution problems with the enzyme reagents/products. The present study for the first time achieved chromatographic separation of analyte peaks from interferences to allow accurate quantitation of normal deoxynucleosides and hydroxymethyldeoxynucleosides of human placental DNA after exposure to FA (Fig. 2). The absolute accuracy of the method was verified by comparing the slopes of N^6-dA and N^2-dG indirect standardizations with those directly obtained with collected solid of known purity by a methanol/water gradient from the HPLC column. N^4-dC cannot be separated from dC when methanol/water is used as mobile phase instead of methanol/ammonium acetate buffer. Since the response factors or slopes for collected N^6-dA and N^2-dG were shown to be not significantly different ($p \leq 0.05$) relative to those obtained indirectly, it is probably true for N^4-dC, especially since only one modified deoxynucleoside peak was detected for reacted dC.

The reaction of FA and guanosine (G) at pH 6.3, 75°C for 48 h at 5-fold excess of FA has been reported to yield a small amount of 7-[7-(β-D-ribofuranosyl)-purin-6(7H)-on-2-yl]-3-(β-D-ribofuranosyl)-5,6,7,8,-tetrahydro-*sym*-triazino[1,2-α]purin-10(3H)-one (Kennedy *et al.*, 1996). The retention time was about 1.75 times that of G. Another modified nucleoside, bis-N^2-guanosinylmethane was reported and 7-[7-(β-D-ribofuranosyl)-purin-6(7H)-on-2-yl]-3-(β-D-ribofuranosyl)-5,6,7,8,-tetrahydro-*sym*-triazino[1,2-α]purin-10(3H)-one increased at pH 10.1. Both compounds have the purine ring, which is very UV sensitive at 254 nm. We repeated experiments with dG at the same conditions, but only two UV-absorbing peaks—dG and N^2-dG—were detected for each injection with other peaks <1% of the hydroxymethyl derivative. Thus dG does not form the other products that G can. This makes dG use advantageous relative to G as a biomarker. Feldman (1973) also reported condensation products when excess DNA base was present. A very recent paper reported that excess FA in 0.1 M phosphate buffer (pH 7.0) produced N^2-dG from from dG, but that the cross-linked dimer was produced as reaction time was increased to 140 h (Cheng *et al.*, 2003). Products of base ring –N–H substitution were not found in our study with deoxynucleosides. These results indicate that the hydromethyl derivative is the only major one formed when FA reacts with dG under our conditions of synthesis.

For the pure N^6-dA and N^2-dG isolated by column collection using a methanol–water elution gradient, the equilibrium constants for formation of hydroxymethyl derivative were calculated to be 74 ± 12. This contrasts with values of 11–17 reported by Feldman (1973) for DNA bases and nucleotides. The thermal stability of these products at room temperature was poor, however, and they must be stored at –20°C or less (Fig. 5).

There was no significant difference at $p \leq 0.05$ between the moles of A and T, or G and C for untreated DNA, which is the expected result. The slope of the normal deoxynucleoside is not significantly different at $p \leq 0.05$ from that of its modified deoxynucleoside, as also expected. The determined (G + C) content is consistent with the mammalian DNA content range of 39–46% (Voet and Voet, 1990). Feldman (1973) reported that single-stranded or denatured double-stranded DNA reacted with formalin to form both cross-links and hydroxymethyl derivatives. Cheng *et al.* (2003) recently reported the presence of both cross-links and hydroxylmethyl derivatives at high dG and dA concentrations and much longer incubation times than used in our synthesis studies.

Some commercially available deoxyadenosines or deoxyguanosines were found to be contaminated with up to 40% adenine or guanine respectively. The quality control of lots from the same manufacturer also often varied. Thus analysis of standards is essential quality assurance/quality control. In the same manner, different commercial enzyme preparations were also evaluated. Type III-S bacterial alkaline phosphatase gave very high blanks with additional peaks that appeared at 17–23 min that interfered with the quantitation of dC. This phosphatase also produced more adenine, guanine and cytosine, lowering the yields of the corresponding deoxynucleosides. Human placental alkaline phosphatase (type XVII) was up to twice as efficient at producing deoxynucleosides as the bacterial alkaline phosphatases (type III, III-L, III-S) and without further degradation products. Some bacterial alkaline

phosphatases also produced large amounts of free bases. The DNA samples also contained late-eluting HPLC peaks that necessitated column cleaning before the next injection. The commercial substrates and enzymes that were most reliable are specified in the Chemicals section.

A number of animal and human studies have shown a link between exposure to FA and DPC (Casanova *et al.*, 1994, 1996; Shaham *et al.*, 2003). Casanova *et al.* (1994) conducted experiments with rats and monkeys and found that rate of DPC formation was proportional to the tissue concentration of FA. DPC in peripheral blood lymphocytes of workers exposed to high FA as biomarkers has also been advocated by Shaham *et al.* (2003). However, peripheral blood lymphocytes are not the target cells for cancer, these being nasal cells in rats and humans (Swenberg *et al.*, 1980, 1983; Kerns *et al.*, 1983; Olsen and Asnaes, 1986; Hansen and Olson, 1995). An endogenous biomarker background also makes it difficult to detect lymphocyte DPC after exposure to low FA air concentrations. DPC is not a unique biomarker for FA and exposure to compounds other than FA, such as acetaldehyde, Cr(VI) and hydroxyl radicals could also result in DPC (Feron *et al.*, 1991). The hydroxymethyldeoxynucleoside markers of effect are therefore certainly more selective than the DPC assays for FA. We are presently applying the technique to nasal cell cultures.

The main advantage of this optimized method is that it offers sensitivity, selectivity, precision and accuracy for the simultaneous determination of all four normal and three modified deoxynucleosides with the most frequently used HPLC detector at 254 nm. This method can be used for routine quantitative analysis at LQLs of about 10 pmol/10 μL for dA, dG, dC, N^6-dA and N^2-dG, and of 22 pmol/10 μL for N^4-dC (Table 1). This sensitivity has potential for use after FA exposure to human tissue.

Acknowledgements

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