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Determination of Oxidative DNA Base Damage by Gas Chromatography-Mass Spectrometry. Effect of Derivatization Conditions on Artifactual Formation of Certain Base Oxidation Products

TIMOTHY G. ENGLAND, ANDREW JENNER*, OKEZIE I. ARUOMA and BARRY HALLIWELL

International Antioxidant and Neurodegenerative Disease Research Centres, Pharmacology Group, University of London King's College, Manresa Road, London, SW3 6LX, UK

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GC-MS is a widely used tool to measure oxidative DNA damage because of its ability to identify a wide range of base modification products. However, it has been suggested that the derivatization procedures required to form volatile products prior to GC-MS analysis can sometimes produce artifactual formation of certain base oxidation products, although these studies did not replicate previously-used reaction conditions, e.g. they failed to remove air from the derivatization vials. A systematic examination of this problem revealed that levels of 8-hydroxyguanine, 8-hydroxyadenine, 5-hydroxycytosine and 5-(hydroxymethyluracil) in commercial calf thymus DNA determined by GC-MS are elevated by increasing the temperature at which derivatization is performed in our laboratory. In particular, 8-hydroxyguanine levels after silylation at 140°C were raised 8-fold compared to derivatization at 23°C. Experiments on the derivatization of each undamaged base revealed that the artifactual oxidation of guanine, adenine, cytosine and thymine respectively was responsible. Formation of the above products was potentiated by not purging with nitrogen prior to derivatization. Increasing the temperature to 140°C or allowing air to be present during derivatization did not significantly increase levels of the other oxidized bases measured.

This work suggests that artifactual oxidation during derivatization is restricted to certain products (8-hydroxyguanine, 8-hydroxyadenine, 5-hydroxycytosine and 5-[hydroxymethyluracil]) and can be decreased by reducing the temperature of the derivatization reaction to 23°C and excluding as much air possible. Despite some recent reports, we were easily able to detect formamidopyrimidines in acid-hydrolyzed DNA. Artifacts of derivatization are less marked than has been claimed in some papers and may vary between laboratories, depending on the experimental procedures used, in particular the efficiency of exclusion of O_2 during the derivatization process.

Keywords: Oxidative stress, DNA damage, GC-MS, 8-hydroxyguanine, 8-hydroxyadenine, 5-hydroxycytosine, 5-hydroxymethyluracil

*Corresponding author. Tel.: +44 171-333-4713. Fax: +44 171-333-4949. E-mail: andrew.jenner@kcl.ac.uk.

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Abbreviations: GS-MS, gas chromatography-mass spectrometry; HPLC-ECD, HPLC with electrochemical detection; FAPy-adenine, 4,6-diamino-5-formamido-pyrimidine; FAPy-guanine, 2,6-diamino-4-hydroxy-5-formamido-pyrimidine; 8-OH guanine, 8-hydroxyguanine; 2-OH adenine, 2-hydroxyadenine; 8-OH adenine, 8-hydroxyadenine; 5-(OH,Me)-uracil, 5-(hydroxymethyl)-uracil; 5-(OH,Me)-hydantoin, 5-hydroxy-5-methylhydantoin; 5-OH hydantoin, 5-hydroxyhydantoin; 5-OH uracil, 5-hydroxy;uracil; 5-OH cytosine, 5-hydroxycytosine

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INTRODUCTION

Reactive oxygen, nitrogen and chlorine species (ROS/RNS/RCS) are generated *in vivo* by various mechanisms including aerobic metabolism, inflammatory responses and exposure to toxins or ionizing radiation (reviewed in Refs. [1–3]). Several such species are capable of causing damage to DNA leading to a variety of DNA lesions such as single and double strand breaks, abasic sites, DNA–protein cross links, sugar modification and chemical changes to both pyrimidine and purine bases.[1–6] The frequency of these lesions and the effectiveness of repair systems could therefore be an important factor in the development of mutagenic and carcinogenic states as well as other degenerative diseases.[3,7–9] Therefore, the accurate assessment of oxidative DNA damage in human samples may be a valuable marker of both the risk of occurrence of pre-carcinogenic events *in vivo* and how such events may be influenced by diet.[9] However, the steady-state levels of base oxidation products in DNA from healthy individuals have yet to be unequivocally established, since the various methodological approaches employed have produced widely differing values between different laboratories.[2,4,9–12].

Methods that have been used include HPLC-ECD measurement of 8-hydroxydeoxyguanosine[2,12] or 8-hydroxyguanine (8-OH guanine)[13] after enzymic or acidic hydrolysis of DNA respectively. Gas chromatography coupled with mass spectrometry is a sensitive method which permits structural identification and measurement of a wide range of base damage products.[4,10,14–16] The pattern of base damage can sometimes be used to identify the ROS/RNS that has attacked DNA, e.g. $^1O_2$, $^{\bullet}OH$, $ONOO^-$ and HOCl give different DNA base damage products.[4,10,17–21] Levels of hydroxylated guanine measured in cellular DNA by GC-MS are often, but not always, greater than those measured by other methods such as HPLC-ECD (reviewed in Refs. [10,11]).

In order for GC-MS to be carried out, DNA is hydrolyzed either by enzymatic means or acid in order to release nucleosides or free bases respectively. Derivatization is subsequently needed to convert the polar nucleosides/bases and internal standards to volatile, thermally stable derivatives which possess characteristic mass spectra.[22,23] The most common derivatization reaction used for analysis of acid hydrolyzed DNA is trimethylsilylation.[4,6,15–23] This reaction is often performed at a high temperature (such as 120°C, although values in certain papers range from 90–140°C) to ensure that the reaction is complete, and air is excluded as far as possible to prevent unnecessary oxidation of samples.[4,10,15–24] If attempts to exclude air are not made and derivatization is carried out at high temperatures, levels of 8-OH guanine and some other oxidized bases can be elevated, apparently due to artifactual oxidation of undamaged base.[25–28] This has led to the suggestion that the higher levels of 8-OH guanine and a few other base modification products detected in DNA by GC-MS are an artifact of the derivatization process.[25–27] However, these studies did not follow usual DNA hydrolysis and derivatization procedures, e.g. they did not exclude air from the derivatization. Thus they are not comparable with previous GC-MS studies which performed derivatization under a nitrogen atmosphere.[4,15–24] The objective of this study was to examine the effect of different derivatization conditions on the analysis of several oxidized DNA bases by GC-MS, to see how far the alleged artifacts could affect the more commonly used derivatization methods as applied in our laboratory.

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METHODS

Calf thymus DNA, 6-azathymine, 2,6-diaminopurine, 8-bromoadenine, 5-hydroxyuracil (isobarbituric acid), 4,6-diamino-5-formamidopyrimidine (FAPy-adenine), 5-(hydroxymethyl) uracil (5-(OH,Me)-uracil), and 2,5,6-triamino-4-hydroxy-pyrimidine were of the highest quality available from Sigma Chemical Company (Poole, Dorset, UK). 8-OH guanine was purchased from Aldrich (Poole, Dorset, UK). 8-Hydroxyadenine (8-OH adenine), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FAPy-guanine) and thymine glycol were synthesized as previously described.[16,20,22,24,29] 2-Hydroxyadenine (2-OH adenine), 5-hydroxycytosine (5-OH cytosine), 5-hydroxyhydantoin (5-OH hydantoin) and 5-hydroxy-5-methyl-hydantoin (5-(OH,Me)-hydantoin) were gifts from Dr. M. Dizdaroglu of the National Institute of Standards and Technology, Maryland, USA. Cellu.Sep dialysis membranes with a relative molecular mass cut off of 3500, silylation grade acetonitrile, and bis(trimethylsilyl)trifluoracetamide (BSTFA) (containing 1% trimethylchlorosilane, TMCS) were obtained from Pierce Chemical Co. (Rockford, IL). Distilled water passed through a purification system (Elga, High Wycombe, Bucks, UK) was used to make up all solutions.

Acid Hydrolysis

Calf thymus DNA (100 µg) containing the internal standards 6-azathymine and 2,6-diaminopurine (0.5 nmol) was hydrolyzed by addition of 0.5 ml of 60% v/v formic acid and heating at 140°C for 45 min in an evacuated, sealed hydrolysis tube. Samples were cooled, lyophilized and then derivatized in poly(tetrafluoroethylene)-capped hypovials (Pierce). One hundred µl of a BSTFA (+1% TMCS)/acetonitrile (4:1 v/v) mixture was added to all samples. Despite some suggestions that hydrolysis with 60% formic acid can completely destroy formamidopyrimidines, we were easily able to measure FAPy-adenine and -guanine in our studies, in agreement with previous literature.[4,16–24]

Efficiency of Derivatization and Derivatization Stability

In preliminary experiments individual aliquots of normal base (100 nmol), damaged base product (1 nmol) or internal standard i.e. 6-azathymine and 2,6-diaminopurine (0.5 nmol), all containing octadecane (100 nmol) were lyophilized from solution. One hundred nmol of normal base is comparable to the level of each base in 100 µg DNA, the amount of DNA used for GC-MS analysis. Samples were derivatized for 1 h at either 140°C, 90°C or 23°C. For each temperature, samples were derivatized under a reduced oxygen concentration using nitrogen gas to displace air prior to capping, otherwise hypovials were capped while open to the air. Octadecane is stable at high temperatures and does not undergo trimethylsilylation. It was therefore used as an inert reference in derivatized samples in order to follow the efficiency of base product derivatization.[28] For each base product only the most complete derivative was monitored e.g. the tetrasilyl derivative of 8-OH guanine.[22,23]

Artifact Formation During Derivatization of Hydrolyzed DNA and Individual Bases

Further investigation of the time course of derivatization showed that formation of derivatives had ceased after 1 h at 90°C and 140°C, but at 23°C 2 h was required. Therefore in separate experiments individual aliquots of hydrolyzed DNA (100 µg), normal base (100 nmol) or damaged base product (1 nmol) were lyophilized from solution and derivatized at different temperatures, 140°C, 90°C and 23°C, with or without nitrogen, for 1 h. Subsequently all samples were analyzed by GC-MS after a further 1 h at 23°C. In order to measure levels of oxidized bases and assess the formation of any artifacts, two different internal standards 6-azathymine and

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2,6 diaminopurine were added to all samples as well as calibration mixtures of authentic standards. Calibration mixtures were made in triplicate (containing 1, 0.5, 0.2, 0.1, 0.02 nmol). Both internal standards are stable during hydrolysis and derivatization. By performing exactly the same derivatization conditions (also hydrolysis conditions for DNA) on the calibration mixtures, the relative molar response factor of each oxidized base to its internal standard can be obtained and used to determine the levels of oxidized bases in different samples under different conditions. 6-Azathymine was used to quantitate the pyrimidine-derived base products, while 2,6-diaminopurine was used for those derived from the purines.

TABLE I Target ions used for GC-MS selected ion monitoring

Base product (trimethylsilyl derivative)	Target ion (m/z)
Thymine	255
5-(OH,Me) hydantoin	331
5-OH hydantoin	317
5-OH uracil	329
5-(OH,Me)-uracil	358
5-OH cytosine	328
Thymine glycol	259
Hypoxanthine	265
FAPy adenine	354
8-OH adenine	352
Xanthine	353
2-OH adenine	352
FAPy guanine	442
Guanine	367
8-OH guanine	440
Azathymine	256
Diaminopurine	351

Analysis of DNA Base Derivatives by Gas Chromatography-Mass Spectrometry

Derivatized samples were analyzed by GC-MS (Hewlett-Packard 5890II gas chromatograph interfaced with a Hewlett-Packard 5971A mass selective detector). The injection port and the GC-MS interface were kept at 250°C and 290°C, respectively. Separations were carried out on a fused silica capillary column (12 m × 0.2 mm i.d.) coated with cross-linked 5% phenylmethylsiloxane (film thickness 0.33 μm) (Hewlett-Packard). Helium was the carrier gas with a flow rate of 0.93 mL/min. Derivatized samples (2.0 μL) were injected into the GC port using a split ratio of 8:1. Column temperature was increased from 125°C to 175°C at 8°C/min after 2 min at 125°C, then from 175°C to 220°C at 30°C/min and held at 220°C for 1 min, and finally from 220°C to 290°C at 40°C/min and held at 290°C for 2 min. Selected-ion monitoring was performed using the electron-ionization mode at 70 eV with the ion source maintained at 189°C. The ions used for quantification for each trimethylsilylated base/damaged base have been previously reported[22,23] and correspond to the molecular ion (M^+) and the characteristic $(M-15)^+$ fragment (Table I).

Quantification of modified bases was achieved by relating the peak area of the compound with the internal standard peak area and applying the following formula:

$$\text{Concentration (nmol/mg DNA)} = A/A_{\text{IST}} \times [\text{IST}] \times (1/K)$$

where K = relative molar response factor for each damaged base, A = peak area of product, A_{IST} = the peak area of the internal standard, and [IST] = concentration of internal standard (5 nmol/mg DNA). K constants were calculated from the slopes of the calibration curves constructed using known concentrations of internal standards and authentic compounds.[19]

Statistical Analysis

Data points are mean ± standard deviation of 5 or more experiments. ANOVA and Student's t-test were carried out as appropriate. Differences of $p < 0.05$ were considered statistically significant.

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RESULTS

Efficiency of Derivatization and Derivatization Stability

Using octadecane as a stable, inert reference,[28] the extent of derivatization of several normal DNA bases and base oxidation products was examined. After incubation for 1 h all bases and both internal standards were derivatized to a lesser degree at lower derivatization temperatures as shown in Figure 1A–C. Generation of derivatives had ceased after 1 h at 90°C or 140°C, but at 23°C their formation did not finish until 2 h. No difference in the extent of derivatization was detected between samples derivatized under air or under a nitrogen atmosphere.

Mixtures of 6-azathymine and 2,6-diaminopurine derivatized alone did not form any other oxidized base derivative and produced highly reproducible, concentration-dependent chromatographic peak areas under constant derivatization conditions. Thus both internal standards fulfilled suitable criteria to act as internal standards, 6-azathymine standardizing the pyrimidines and 2,6-diaminopurine standardizing the purines owing to their respective similar chemical structures.

The time course of the derivatization reaction at 23°C showed that the trimethylsilylation reaction had not finished after 1 h. The maximal formation of silylated base derivatives obtainable at 23°C was achieved after 2 h, suggesting that the reaction was finished, whether the samples had been purged with nitrogen or not.

Artifact Formation During Derivatization of Individual Bases

During derivatization of each oxidized base no other base product was detected at any of the different temperatures, with or without nitrogen purging.[15] For all derivatization conditions, calibration samples exhibited good concentration linearity ($r > 0.97$, range 0.02–1.0 nmol).

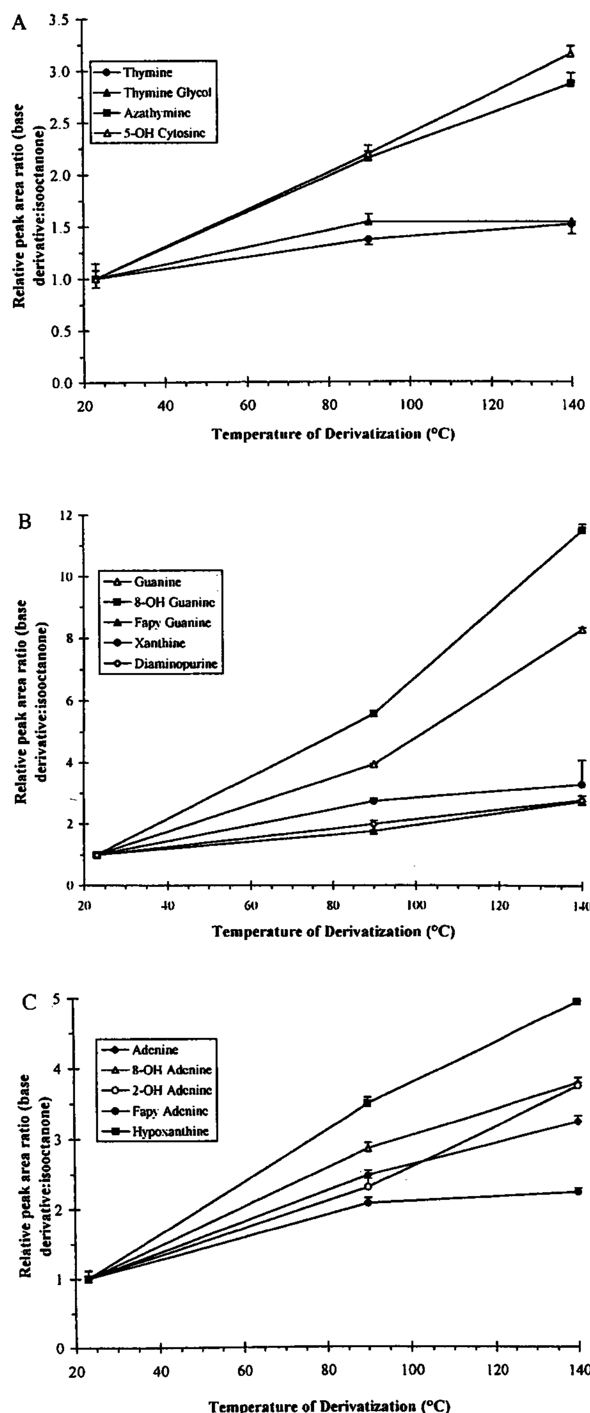

FIGURE 1 Influence of derivatization reaction temperature on extent of derivatization. Compounds were derivatized for 1 h and levels of derivative were calculated by comparison with octadecane. Values at 23°C = 1.0. Data points are means ± S.D. of 5 or more experiments.

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TABLE II Levels of oxidized bases detected by GC-MS (23°C derivatization) in commercial undamaged DNA bases

DNA damaged base (%)	Guanine	Adenine	Cytosine	Thymine
5-(OH,Me) hydantoin	N.D.	N.D.	0.005 ± 0.003	0.004 ± 0.001
5-OH hydantoin	0.38 ± 0.12	N.D.	0.005 ± 0.002	0.005 ± 0.002
5-OH uracil	N.D.	N.D.	0.009 ± 0.002	N.D.
5-(OH,Me)-uracil	N.D.	N.D.	N.D.	0.002 ± 0.001*
5-OH cytosine	0.04 ± 0.01	N.D.	0.025 ± 0.012*	N.D.
Thymine glycol (cis)	N.D.	N.D.	N.D.	0.007 ± 0.001
FAPy adenine	ND.	N.D.	N.D.	N.D.
8-OH adenine	0.11 ± 0.02	0.010 ± 0.05*	N.D.	N.D.
2-OH adenine	N.D.	N.D.	N.D.	N.D.
FAPy guanine	0.06 ± 0.002	N.D.	N.D.	N.D.
8-OH guanine	0.11 ± 0.03*	N.D.	N.D.	N.D.

N.D. = not detected. Data points are means ± S.D. of 5 or more experiments. Bases marked with (*) are affected by derivatization conditions.

Commercial guanine, adenine, cytosine and thymine derivatized at room temperature contain trace, but detectable levels of some oxidized bases (Table II). This is probably due to impurities in the commercial products, in particular guanine, since only traces of these oxidized products were detected.

Knowledge of the temperature effects on derivatization (Figure 1) allows correction for such effects when examining how temperature and air alter the amount of base oxidation products detected. Theoretically therefore, analysis of DNA bases under any derivatization conditions with the appropriate calibration should give the same values for levels of oxidized bases. Indeed this was observed for FAPy-guanine, xanthine, 5-OH hydantoin, 2-OH adenine, FAPy-adenine, hypoxanthine, thymine glycol, 5-(OH,Me)-hydantoin and 5-hydroxyuracil. However, increasing the derivatization temperature under our reaction conditions led to significant increases in the levels of 8-OH guanine (Figure 2A), 8-OH adenine (Figure 2B), 5-OH cytosine (Figure 2C) and 5-(OH,Me)-uracil (Figure 2D) from guanine, adenine, cytosine and thymine respectively, but not the other products listed above.

Increased formation of the above four oxidized bases at high derivatization temperatures was

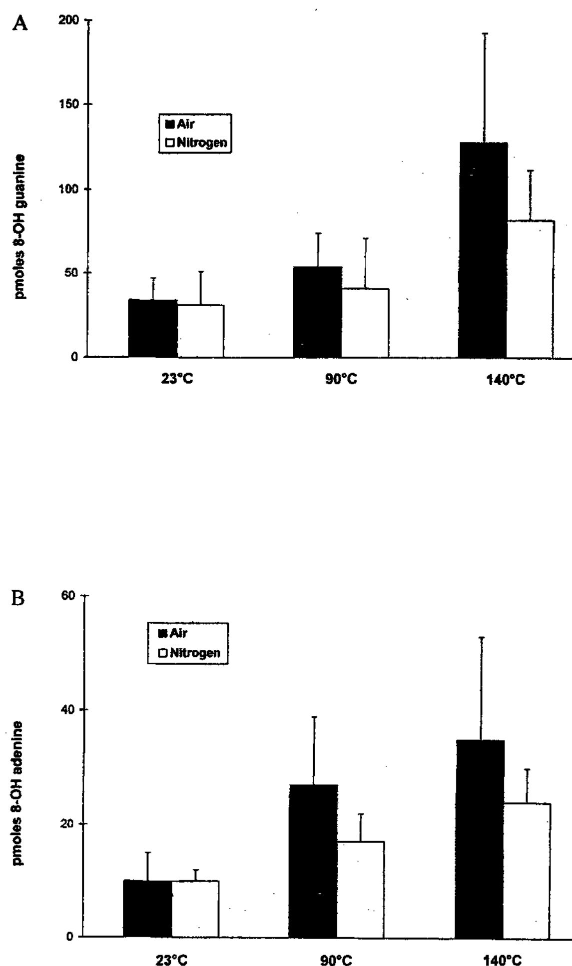

FIGURE 2A and B

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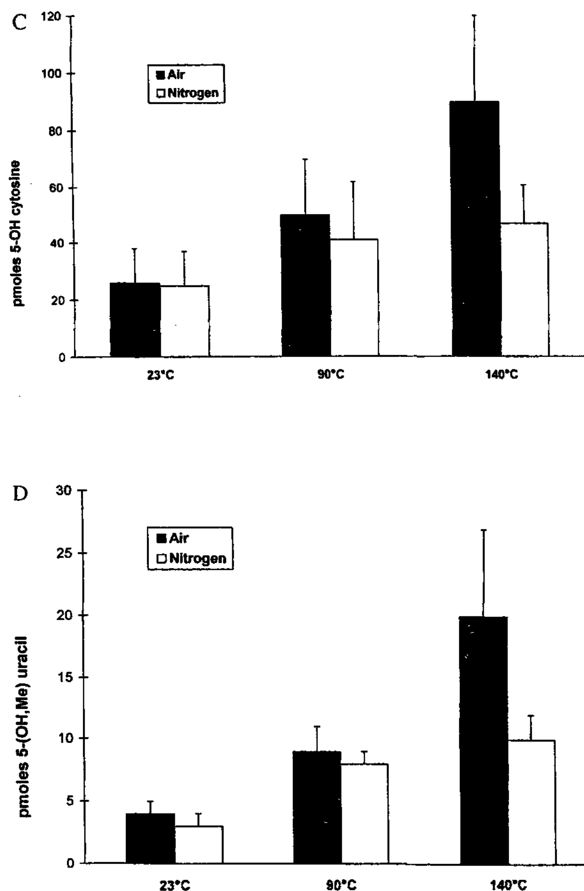

FIGURE 2C and D

FIGURE 2 Influence of derivatization reaction temperature and gaseous atmosphere on levels of 8-OH guanine (A), 8-OH adenine (B), 5-OH cytosine (C) and 5-(OH,Me)-uracil (D) detected in 100 nmol of guanine, adenine, cytosine and thymine respectively. Samples were derivatized at each temperature for 1 h and then kept at 23°C for 1 h prior to GC-MS analysis. Data points are means ± S.D. of 5 or more experiments.

potentiated if samples were not purged with nitrogen prior to derivatization (Figure 2). Nitrogen purging had no effect on the amounts of oxidized bases measured at 23°C.

Artifact Formation During Derivatization of Hydrolysed Calf Thymus DNA

Several oxidized base products could be measured in hydrolyzed calf thymus DNA (Table III). Increasing derivatization temperature resulted in increasing levels of 8-OH guanine, 8-OH adenine, 5-OH cytosine and (5-OH,Me)-uracil but not of any other base product (Figure 3). These increases were not caused by differences in derivatization efficiency, since calibration mixtures of oxidized bases were derivatized under each of the 3 different conditions as explained above. At 140°C with nitrogen purging, 8-OH guanine was increased to 850% that of the 23°C

TABLE III Levels of oxidized bases detected by GC-MS at various derivatization temperatures in hydrolyzed calf thymus DNA

DNA damaged base (pmol mg^{-1} DNA)	23°C	90°C	140°C
5-(OH,Me) hydantoin	39 ± 6	46 ± 8	52 ± 12
5-OH hydantoin	29 ± 5	29 ± 2	44 ± 11
5-OH uracil	51 ± 6	73 ± 6	77 ± 7
5-(OH,Me)-uracil*	22 ± 4	42 ± 13	105 ± 33
5-OH cytosine*	124 ± 3	201 ± 18	491 ± 51
Thymine glycol (*cis*)	141 ± 19	161 ± 21	160 ± 25
FAPy adenine	88 ± 12	126 ± 22	111 ± 24
8-OH adenine*	93 ± 11	195 ± 45	497 ± 81
2-OH adenine	55 ± 8	61 ± 16	68 ± 19
FAPy guanine	124 ± 15	158 ± 32	149 ± 28
8-OH guanine*	94 ± 27	256 ± 75	852 ± 153

Data points are means ± S.D. of 5 or more experiments. Bases marked with (*) are affected by derivatization conditions.

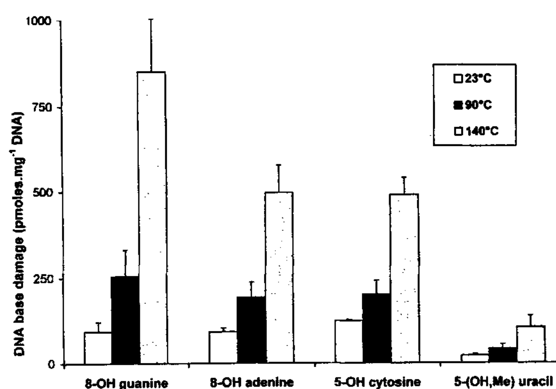

FIGURE 3 Influence of derivatization reaction temperature on the level of 8-OH guanine, 8-OH adenine, 5-OH cytosine and 5-(OH,Me)-uracil detected in hydrolyzed calf thymus DNA by GC-MS. Samples were purged with nitrogen before derivatization at each temperature for 1 h and then kept at 23°C for 1 h prior to GC-MS analysis. Data points are means ± S.D. of 5 or more experiments.

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(nitrogen gassed) level and at 90°C even under nitrogen, levels were twice those of 23°C values. Statistically-significant changes were observed for 8-OH adenine, 5-OH cytosine and 5-(OH,Me)-uracil (Figure 3).

DISCUSSION

Quantitation of pure base oxidation products following trimethylsilylation by GC-MS (SIM) analysis was found to be highly sensitive and reliable in agreement with much previous work.[4,15,16,19–24] Figure 4 shows a profile of the ion at m/z 440 for 8-OH guanine. For all derivatization conditions, calibration samples exhibited good concentration linearity ($r > 0.97$, range 0.02–1.0 nmol) and no other products were generated during derivatization.

The larger chromatographic peaks at higher temperatures of derivatization would usually encourage the use of heat to promote the reaction. Indeed, the optimal incubation time of 2 h at 23°C contrasts with that of 20 min required for the same reaction at 130°C.[16] Even after 2 h at 23°C, derivatization may well be incomplete, although formation of further base derivatives had stopped. Although the derivatized samples are exposed to 250°C during sample injection, the possibility of further oxidation is minimal since dwell time is only a few seconds and the injection port is constantly purged with helium that has passed through chemical filters to remove oxygen.

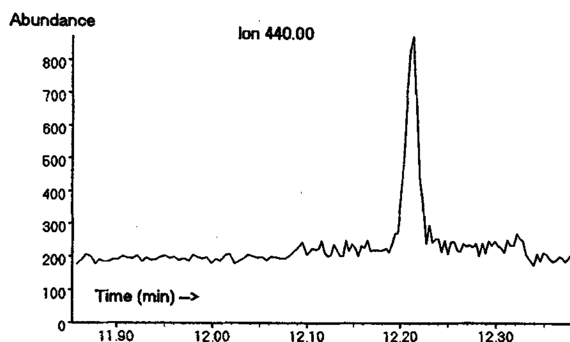

FIGURE 4 Typical selective ion chromatogram of 8-OH guanine (tetrakis-Me_3Si derivative) in calf thymus DNA (100 µg). After acid hydrolysis the sample was derivatized at 23°C for 2 h and approximately 0.25% was injected (2 µL; 1:8 split) for GC-MS analysis.

This study shows that 8-OH guanine, 8-OH adenine, 5-OH cytosine and to a lesser extent, (5-OH,Me)-uracil (Figure 3) can be artifactually increased during derivatization of individual DNA bases or hydrolyzed DNA, at high temperatures under the conditions used in our laboratory. Previous studies reporting artifacts failed to exclude air,[25–27] although heating DNA bases in the presence of oxygen is obviously likely to cause oxidation.[9] Indeed the highest level of 8-OH guanine detected in calf thymus DNA by GC-MS (2426 pmol/mg DNA) was obtained when air was not excluded from derivatization at 130°C for 30 min.[25]

However, our data show that artifacts can occur even with N_2 purging, a technique widely used to exclude air during derivatization. Differences in terms of the magnitude of artifact between this and previous studies,[25–28] particularly with 8-OH guanine, are probably due to differences in the amount and types of derivatization solvents used, as well as the time, temperature and/or failure to even attempt to remove air as in some earlier studies.

The observation that the levels of 8-OH guanine, 8-OH adenine, 5-OH cytosine and 5-(OH,Me)-uracil were still elevated by temperature when samples were purged with nitrogen suggests that oxygen was not completely removed. Of course, more rigorous removal of O_2, e.g. evacuation and/or purging with helium might have achieved still lower results, but it may prove difficult to completely and reproducibly exclude air from samples. In addition, it is unlikely that artefactual oxidation is completely prevented even using derivatization at 23°C under N_2.[30] This is perhaps suggested by the fact that our numbers for levels of base damage products in calf thymus DNA are not the lowest of those in the literature. Levels of 5-OH cytosine,

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8-OH adenine and 8-OH guanine in some previous studies (for example Refs. [31–34]) using high-temperature derivatization are comparable to values obtained at room temperature in our studies (indicating that effectiveness of artifact prevention may vary between laboratories).

The data in this paper suggest that artefactual product formation applies only to a few base products and can be minimized by derivatization of hydrolyzed DNA at room temperature after N_2 flushing. This paper also shows that most bases, including 8-OH guanine, 8-OH adenine and thymine glycol, take longer to be derivatized at room temperature and for maximal sensitivity samples ought to be incubated for at least 2 h. Although trifluoroacetic acid (TFA) has been reported to improve room temperature derivatization of guanine and 8-OH guanine,[28] we have found that TFA causes inconsistent derivatization of some pyrimidine oxidation products, which adversely affects their accurate quantitation.

Recent developments in the application of GC/MS to the measurement of oxidized DNA bases have included the use of isotope dilution (GC/IDMS),[15,28,35,36] a valuable technique for establishing absolute levels of base damage products in DNA. This technique was not used in the work described in this paper to save costs and because inclusion of isotope dilution will not affect comparative studies of hydrolysis and derivatization conditions, nor would any changes during the acidic hydrolysis stage. Nevertheless, improvement of assay conditions for GC-MS is warranted because of the ability of this technique to measure a range of DNA damage products.[4,9,20] Indeed several cases are now known where measurement of a single DNA base product can give misleading results.[37–41] For example, rises in 8-OH guanine in Parkinson's disease may not have been reflective of rises in total DNA oxidative damage.[37] Supplementation of healthy human subjects with excess vitamin C can raise levels of certain oxidative DNA damage products in white cells,[38–40] an effect that would have been missed from measurements of 8-OH guanine alone.

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References

[1] B. Halliwell and J.M.C. Gutteridge (1998) *Free Radicals in Biology and Medicine* 3rd edition, Clarendon Press, Oxford, UK.

[2] R.A. Floyd (1990) The role of 8-hydroxyguanine in carcinogenesis. *Carcinogenesis* **11**, 1447–1450.

[3] H. Wiseman and B. Halliwell (1996) Damage to DNA by reactive oxygen and nitrogen species – role in inflammatory disease and progression to cancer. *Biochemical Journal* **313**, 17–29.

[4] M. Dizdaroglu (1990) Gas-chromatography mass-spectrometry of free radical-induced products of pyrimidines and purines in DNA. *Methods in Enzymology* **193**, 842–857.

[5] C. von Sonntag (1987) *The Chemical Basis of Radiation Biology*, Taylor and Francis, London.

[6] Z. Nackerdien, G. Rao, M.A. Cacciuttolo, E. Gajewski and M. Dizdaroglu (1991) Chemical nature of DNA–protein cross-links produced in mammalian chromatin by hydrogen peroxide in the presence of iron or copper ions. *Biochemistry* **30**, 4872–4879.

[7] L.A. Loeb (1989) Endogenous carcinogenesis: molecular oncology into the 21st century – Presidential address. *Cancer Research* **49**, 5489–5496.

[8] B. Singer and B. Hang (1997) What structural features determine repair enzyme specificity and mechanism in chemically modified DNA? *Chemical Research in Toxicology* **10**, 713–732.

[9] B. Halliwell (1996) Oxidative stress, nutrition and health. Experimental strategies for optimization of nutritional antioxidant intake in humans. *Free Radical Research* **25**, 57–74.

[10] B. Halliwell and M. Dizdaroglu (1992) The measurement of oxidative damage to DNA by HPLC and GC-MS techniques. *Free Radical Research Communications* **16**, 75–87.

[11] A. Collins, J. Cadet, B. Epe and C. Gedik (1997) Problems in the measurement of 8-oxoguanine in human DNA. Report of a workshop, DNA Oxidation, held in Aberdeen, UK, 19–21 January, 1997. *Carcinogenesis* **18**, 1833–1836.

[12] M.K Shigenaga and B.N. Ames (1991) Assays for 8-hydroxy-2′-deoxyguanosine – a biomarker of *in vivo* oxidative DNA damage. *Free Radical Biology and Medicine* **10**, 211–216.

[13] H. Kaur and B. Halliwell (1996) Measurement of oxidized and methylated DNA bases by HPLC with electrochemical detection. *Biochemical Journal* **318**, 21–23.

[14] L. Marnett and P.C. Burcham (1993) Endogenous DNA adducts: potential and paradox. *Chemical Research in Toxicology* **6**, 771–785.

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[15] M. Dizdaroglu (1993) Quantitative-determination of oxidative base damage in DNA by stable isotope-dilution mass-spectrometry. *FEBS Letters* **315**, 1–6.
[16] A.F. Fuciarelli, B.J. Wegher, E. Gajewski, M. Dizdaroglu and W.F. Blakely (1989) Quantitative measurement of radiation-induced base products in DNA using gas-chromatography mass-spectrometry. *Radiation Research* **119**, 219–231.
[17] J.P.E. Spencer, J. Wong, A. Jenner, O.I. Aruoma, C.E. Cross and B. Halliwell (1995) Base modifications and strand breakage in isolated calf thymus DNA and in DNA from human skin epidermal keratinocytes exposed to peroxynitrite or 3-morpholinosydnonimine. *Chemical Research in Toxicology* **9**, 1152–1158.
[18] M. Whiteman, A. Jenner and B. Halliwell (1997) Hypochlorous acid-induced base modifications in isolated calf thymus DNA. *Chemical Research in Toxicology* **10**, 1240–1246.
[19] A.F. Fuciarelli, B.J. Wegher, W.F. Blakely and M. Dizdaroglu (1990) Yields of radiation-induced base products in DNA – effects of DNA conformation and gassing conditions. *International Journal of Radiation Biology* **58**, 397–415.
[20] O.I. Aruoma, B. Halliwell and M. Dizdaroglu (1989) Iron ion dependent modification of bases in DNA by the superoxide radical-generating system hypoxanthine/xanthine oxidase. *Journal of Biological Chemistry* **264**, 13024–13028.
[21] O.I. Aruoma, B. Halliwell, B. Gajewski and M. Dizdaroglu (1991) Copper-ion-dependent damage to the bases in DNA in the presence of hydrogen peroxide. *Biochemical Journal* **273**, 601–604.
[22] M. Dizdaroglu (1984) The use of capillary gas chromatography-mass spectrometry for identification of radiation-induced DNA base damage and DNA base-amino acid crosslinks. *Journal of Chromatography* **295**, 103–121.
[23] M. Dizdaroglu (1985) Application of capillary gas chromatography-mass spectrometry to chemical characterization of radiation-induced base damage of DNA: implications for assessing DNA repair processes. *Analytical Biochemistry* **144**, 593–603.
[24] M. Dizdaroglu and D.S. Bertgold (1986) Characterization of free-radical induced base damage in DNA at biologically relevant levels. *Analytical Biochemistry* **156**, 182–188.
[25] J.-L. Ravanat, R.J. Turesky, E. Gremaud, L.J. Trudle and R.H. Stadler (1995) Determination of 8-oxoguanine in DNA by gas-chromatography mass-spectrometry and HPLC-electrochemical detection – overestimation of the background level of the oxidized base by the gas-chromatography mass-spectrometry assay. *Chemical Research in Toxicology* **8**, 1039–1045.
[26] T. Douki, T. Delatour, F. Bianchini and J. Cadet (1996) Observation and prevention of an artifactual formation of oxidized DNA bases and nucleosides in the GC-EIMS method. *Carcinogenesis* **17**, 347–353.
[27] J.-L. Ravanat, T. Douki, R. Turesky and J. Cadet (1997) Measurement of oxidized bases in DNA. Comparison between HPLC-EC and GC-MS assays. *Journal De Chimie Physique Et De Physico-Chimie Biologique* **94**, 306–312.
[28] M. Hamberg and L.Y. Zhang (1995) Quantitative-determination of 8-hydroxy-guanine and guanine by isotope-dilution mass-spectrometry. *Analytical Biochemistry* **229**, 336–344.
[29] M. Dizdaroglu, E. Holwitt, M.P. Hagen and W.F. Blakely (1986) Formation of cytosine glycol and 5,6-dihydroxycytosine in deoxyribonucleic acid on treatment with osmium tetroxide. *Biochemical Journal* **235**, 531–536.
[30] A. Jenner, T.G. England, O.I. Aruoma and B. Halliwell (1998) Measurement of oxidative DNA damage by gas chromatography-mass spectrometry: ethanethiol prevents artifactual generation of oxidized DNA bases. *Biochemical Journal* **331**, 365–369.
[31] T.H. Zastawny, S.A. Altman, L. Randers-Eichhorn, R. Madurawe, J.A. Lumpkin, M. Dizdaroglu and G. Rao (1995) DNA base modifications and membrane damage in cultured mammalian cells treated with iron ions. *Free Radical Biology and Medicine* **18**, 1013–1022.
[32] P.K. Liu, C.Y. Hsu, M. Dizdaroglu, R.A. Floyd, Y.W. Kow, A. Karakaya, L. Rabow and J.-K. Ciu (1996) Damage, repair and mutagenesis in nuclear genes of the brain after forebrain ischemia and reperfusion. *Journal of Neuroscience* **16**, 6795–6806.
[33] Z. Nackerdien, R. Olinski and M. Dizdaroglu (1992) DNA base damage in chromatin of γ-irradiated cultured human cells. *Free Radical Research Communications* **16**, 259–272.
[34] T. Mori and M. Dizdaroglu (1994) Ionizing radiation causes greater DNA base damage in radiation-sensitive mutant M10 cells than in parent mouse lymphoma L5178Y cells. *Radiation Research* **140**, 85–90.
[35] C.J. LaFrancois, J. Fujimoto and L.C. Sowers (1998) Synthesis and chemical characterization of isotopically enriched pyrimidine deoxy-nucleoside oxidation damage products. *Chemical Research in Toxicology* **11**, 75–83.
[36] I.D. Podmore, M.S. Cook, K.E. Herbert and J. Lunec (1996) Quantitative determination of cyclobutane thymine dimers in DNA by stable isotope dilution mass spectrometry. *Photochemistry and Photobiology* **64**, 310–315.
[37] Z.I. Alam, A. Jenner, A.J. Lees, N. Cairns, C.D. Marsden, P. Jenner and B. Halliwell (1997) Oxidative DNA damage in the Parkinsonian brain: an apparent selective increase in 8-hydroxyguanine levels in substantia nigra. *Journal of Neurochemistry* **69**, 1196–1203.
[38] I. Podmore, H.R. Griffiths, K.E. Herbert, W. Mistry, P. Mistry and J. Lunec (1998) Vitamin C exhibits pro-oxidant properties. *Nature* **392**, 559–560.
[39] A. Rehman, C.S. Collis, M. Yang, M. Kelly, A.T. Diplock, B. Halliwell and C. Rice-Evans (1998) Effects of iron and vitamin C co-supplementation on oxidative damage to DNA in healthy volunteers. *Biochemical and Biophysical Research Communications* **246**, 293–298.
[40] B. Halliwell, T.G. England, A. Jenner, O.I. Aruoma, A. Rehman, C. Rice-Evans, C. Geissler, M. Whiteman, J.P.E. Spencer and E. Beatty (1998) Oxidative DNA damage in human cancer and its modulation by dietary constituents: *Human Experimental Toxicology* (in press).
[41] H. Faure, M. Mousseau, J. Cadet, C. Guimier, M. Tripier, H. Hida and A. Favier (1998) Urine 8-oxo-7,8-dihydro-2′-deoxyguanosine vs 5-(hydroxymethyl)uracil as DNA oxidation markers in adriamycin-treated patients. *Free Radical Research* **28**, 377–382.

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