Mass Spectrometric Assays for the Tandem Lesion 8,5′-Cyclo-2′-deoxyguanosine in Mammalian DNA

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Received December 3, 2001; Revised Manuscript Received January 24, 2002

ABSTRACT: 8,5′-Cyclopurine 2′-deoxynucleosides are among the major lesions in DNA that are formed by attack of hydroxyl radical. These compounds represent a concomitant damage to both sugar and base moieties of the same nucleoside and thus can be considered tandem lesions. Because of the presence of a covalent bond between the sugar and purine moieties, these tandem lesions are not repaired by base excision repair but by nucleotide excision repair. Thus, they may play a role in diseases with defective nucleotide excision repair. We recently reported the identification and quantification of 8,5′-cyclo-2′-deoxyadenosine (8,5′-cdAdo) in DNA by liquid chromatography/mass spectrometry with the isotope dilution technique (LC/IDMS) [Dizdaroglu, M., Jaruga, P., and Rodriguez, H. (2001) *Free Radical Biol. Med. 30*, 774−784]. In the present work, we investigated the measurement of 8,5′-cyclo-2′-deoxyguanosine (8,5′-cdGuo) in DNA by LC/IDMS. A methodology was developed for the separation of both (5′*R*)- and (5′*S*)-diastereomers of this compound in enzymic hydrolysates of DNA. The mass spectra were recorded using an atmospheric pressure ionization−electrospray process in the positive ionization mode. For quantification, stable isotope-labeled analogues of (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo were prepared and isolated by semipreparative LC to be used as internal standards. The sensitivity level of LC/MS in the selected ion monitoring mode (LC/MS−SIM) was determined to be approximately 15 fmol of these compounds on the LC column. The yield of 8,5′-cdGuo was measured in DNA exposed in aqueous solution to ionizing radiation at doses from 2.5 to 40 Gy. For comparison, gas chromatography/mass spectrometry with the isotope dilution technique (GC/IDMS) was also employed to measure both (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo in DNA. Both techniques yielded nearly identical results. The radiation chemical yield of 8,5′-cdGuo was similar to those of other major purine-derived lesions in DNA. The sensitivity level of GC/MS−SIM was determined to be significantly greater than that of LC/MS−SIM (1 vs 15 fmol). The background levels of (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo were measured in calf thymus DNA and in DNA samples isolated from three different types of cultured human cells. The levels of (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo were approximately 2 lesions/10^6 DNA nucleosides and 10 lesions/10^6 DNA nucleosides, respectively. No significant differences between tissues were observed in terms of these background levels. The results showed that both LC/IDMS and GC/IDMS are well suited for the sensitive detection and precise quantification of both (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo in DNA.

Free radicals, most notable hydroxyl radical (•OH),[1] react with DNA constituents at or near diffusion-controlled rates (reviewed in ref *1*). Abstraction of H atoms from the C−H bonds of the sugar moiety or from the methyl group of thymine and addition to the double bonds of the heterocyclic bases generate sugar and base radicals (reviewed in refs *1*−*3*). Further reactions of these radicals produce base and sugar lesions, strand breaks, DNA−protein cross-links, and 8,5′-cyclopurine 2′-deoxynucleosides (reviewed in refs *2* and *3*). The latter products arise from the attack of the C-5′ centered radical of the sugar moiety at the C-8 of the purine within the same nucleoside leading to intramolecular cyclization, followed by oxidation of the thus formed N-7 centered radical (*4*). These compounds are considered tandem lesions because of concomitant damage to both sugar and base moieties of the same nucleoside. The cyclization reaction was first observed in adenosine 5′-monophosphate (AMP) upon •OH attack, giving rise to 8,5′-cyclo-AMP (*4*). Subsequent studies showed the formation of 8,5′-cyclo-2′-

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[1] Abbreviations: •OH, hydroxyl radical; AMP, adenosine 5′-monophosphate; 8,5′-cdAdo, 8,5′-cyclo-2′-deoxyadenosine; 8,5′-cdGuo, 8,5′-cyclo-2′-deoxyguanosine; BER, base excision repair; NER, nucleotide excision repair; LC/IDMS, liquid chromatography/isotope dilution mass spectrometry; GC/IDMS, gas chromatography/isotope dilution mass spectrometry; dGTP, 2′-deoxyguanosine 5′-triphosphate; API-ES, atmospheric pressure ionization−electrospray; MH^+, protonated molecular ion; MNa^+, sodium adduct ion; $M^{+\bullet}$, molecular ion; 8-OH-dGuo, 8-hydroxy-2′-deoxyguanosine; 8-OH-dAdo, 8-hydroxy-2′-deoxyadenosine; FapyGua, 2,6-diamino-4-hydroxy-5-formamidopyrimidine; SIM, selected ion monitoring.

10.1021/bi016004d CCC: $22.00
Published on Web 02/19/2002

deoxyadenosine (8,5′-cdAdo) in dAMP, poly(adenylic acid), and DNA (*5*−*12*) and the formation of 8,5′-cyclo-2′-deoxyguanosine (8,5′-cdGuo) in DNA in vitro and in human cells exposed to ionizing radiation (*13*, *14*). Both the (5′*R*)- and (5′*S*)-diastereomers of 8,5′-cdAdo and 8,5′-cdGuo were observed.

From the point of view of DNA repair, 8,5′-cyclopurine 2′-deoxynucleosides might not be repaired by base excision repair (BER) because of the presence of a covalent bond between the sugar and base moieties of the same nucleoside. However, they might be repaired by nucleotide excision repair (NER), as was previously suggested (*13*, *14*). Indeed, recent studies showed that one of these tandem lesions, 8,5′-cdAdo, was repaired by NER and not by BER in mammalian cells, with the repair of (5′*R*)-8,5′-cdAdo being more efficient than that of (5′*S*)-8,5′-cdAdo (*15*, *16*). Furthermore, it was found that 8,5′-cdAdo was a strong block to gene expression and poorly repaired in NER-deficient cells (*15*). This suggests that 8,5′-cyclopurine 2′-deoxynucleosides might accumulate in DNA repair-deficient cells and might lead to significant pathological consequences. The role of 8,5′-cyclopurine 2′-deoxynucleosides in biological consequences of oxidative damage to DNA is largely unknown. Recent synthesis of oligonucleotides containing 8,5′-cdAdo and 8,5′-cdGuo (*17*, *18*) might facilitate the investigation of the biological effects of these compounds. It would be important to identify and quantify these compounds in cells for an understanding of their role in diseases.

Recently, we reported the measurement of 8,5′-cdAdo in DNA by liquid chromatography/isotope dilution mass spectrometry (LC/IDMS) and gas chromatography/isotope dilution mass spectrometry (GC/IDMS) (*19*). A ^{32}P-postlabeling assay for this lesion was also described (*20*). In the present work, we studied the identification and quantification of 8,5′-cdGuo in DNA by LC/IDMS and GC/IDMS.

EXPERIMENTAL PROCEDURES

Materials.[2] Calf thymus DNA, 2′-deoxyguanosine (dGuo), guanine, nuclease P1, phosphodiesterase I, and phosphodiesterase II were obtained from Sigma Chemical Co. (St. Louis, MO). Alkaline phosphatase was purchased from Roche Diagnostics Corp. (Indianapolis, IN). 2′-Deoxyguanosine 5′-triphosphate-1,3,7,9-$^{15}N_4$-(2-amino-^{15}N) (dGTP-$^{15}N_5$) was purchased from Medical Isotopes, Inc. (Pelham, NH). Acetonitrile (HPLC grade) was from Burdick and Jackson (Muskegon, MI). Biomax5 ultrafiltration membranes (molecular mass cutoff of 5 kDa) were purchased from Millipore (Bedford, MA). Water (HPLC grade) for LC/MS analyses was from J. T. Baker (Phillipsburg, NJ). Water purified through a Milli-Q system (Millipore, Bedford, MA) was used for all other applications.

Cell Culture, DNA Isolation, and Preparation of γ-Irradiated DNA Samples. HeLa cells (ATCC, Manassas, VA) were placed in antibiotic-free Dulbecco's modified Eagle medium (Life Technologies, Rockville, MD) containing 10% (v/v) fetal bovine serum (Life Technologies, Rockville, MD) and grown in 175 cm^2 flasks at 37 °C in a humidified cell culture incubator with 5% CO_2/95% air. Medium was aspirated off, and cells were rinsed with 20 mL of 1× PBS. Cells were detached by adding 3 mL of a trypsin/EDTA solution (Life Technologies, Rockville, MD). Human dermal neonatal fibroblast cells (Cascade Biologics, Portland, OR) were cultured in medium 106 supplemented with low serum growth supplement (LGGS) in the absence of antibiotics and antimycotics (Cascade Biologics, Portland, OR). Medium was aspirated off, and cells were rinsed and detached by adding 3 mL of a trypsin/EDTA solution (Cascade Biologics, Portland, OR). The trypsin/EDTA solution was immediately removed, and an aliquot of 3 mL of a trypsin neutralizer solution (Cascade Biologics, Portland, OR) was added to each flask. Human epidermal neonatal keratinocyte cells (Cascade Biologics, Portland, OR) were cultured in EpiLife medium supplemented with human keratinocyte growth supplement (HKGS) in the absence of antibiotics and antimycotics and then treated as above. DNA was isolated from cells using a blood and cell culture DNA maxi kit (Qiagen, Valencia, CA). DNA was recovered by spooling, washed once in 70% ethanol, and then air-dried. DNA was dissolved in 10 mM sodium phosphate buffer at a concentration of 0.3 mg/mL.

Calf thymus DNA was dissolved in 10 mM sodium phosphate buffer (pH 7.4) (0.3 mg/mL) at 4 °C. Aliquots of the DNA solution were bubbled with N_2O and irradiated with γ-rays in a ^{60}Co γ-source at doses of 2.5, 5, 10, 20, and 40 Gy (dose rate 30 Gy/min). Unirradiated and irradiated calf thymus DNA samples and those isolated from cultured cells were dialyzed against water for 18 h at 4 °C. Water outside the dialysis bags was changed three times during the course of the dialysis. Subsequently, the DNA concentration was measured by UV spectroscopy and by GC/IDMS. For the measurement by GC/IDMS, aliquots of DNA samples containing a known amount of dGuo-$^{15}N_5$ as an internal standard were hydrolyzed with formic acid, trimethylsilylated, and then analyzed as described below. Upon hydrolysis, dGuo-$^{15}N_5$ yields guanine-$^{15}N_5$, which is used as an internal standard to quantify guanine in DNA by GC/IDMS and consequently to determine the DNA amount. The measurements by GC/IDMS and by UV spectroscopy yielded similar results. Subsequently, aliquots of DNA solutions containing 100 μg of DNA were dried under vacuum in a SpeedVac.

Preparation of Stable Isotope-Labeled Analogues of (5′R)-8,5′-cdGuo and (5′S)-8,5′-cdGuo. Stable isotope-labeled analogues of (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo were not available. To prepare these compounds, an aqueous solution of commercially available dGTP-$^{15}N_5$ (5 mg/100 mL) was prepared and then bubbled with N_2O and exposed to ionizing radiation in a ^{60}Co γ-source at a dose of 400 Gy (dose rate 30 Gy/min). For comparison, an aqueous solution of dGuo (0.1 mM) was bubbled with N_2O and irradiated at the same dose. This treatment is known to produce both (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo from dGuo (*13*). Thus, it was expected that both (5′*R*)-8,5′-cdGTP-$^{15}N_5$ and (5′*S*)-8,5′-cdGTP-$^{15}N_5$ would be formed from dGTP-$^{15}N_5$. To desphosphorylate these compounds, an aliquot of 100 mL of the irradiated solution of dGTP-$^{15}N_5$ was lyophilized to dryness, dissolved in 1 mL of 10 mM phosphate buffer (pH 8.0), and

[2] Certain commercial equipment or materials are identified in this paper in order to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

incubated with alkaline phosphatase (5 units) at 37 °C for 24 h. The samples were filtered using ultrafiltration membranes with a molecular mass cutoff of 5 kDa by centrifugation at 6000*g* for 30 min. An aliquot (5 μL) of the filtered samples was analyzed by LC/MS and found to contain both (5′*R*)-8,5′-cdGuo-$^{15}N_5$ and (5′*S*)-8,5′-cdGuo-$^{15}N_5$. Another aliquot was lyophilized, trimethylsilylated, and analyzed by GC/MS. This analysis confirmed the presence of both (5′*R*)-8,5′-cdGuo-$^{15}N_5$ and (5′*S*)-8,5′-cdGuo-$^{15}N_5$ on the basis of the previously reported GC/MS analysis of these compounds (*13*). Subsequently, (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo were isolated by semipreparative LC. The details of these LC/MS and GC/MS analyses and the isolation of both compounds are described in the Results section.

Enzymic Hydrolysis of DNA to Nucleosides. Hydrolysis of DNA was performed using three different procedures, which had previously been described (*21*−*23*). In the case of LC/MS, all three procedures were suitable for the measurement of modified nucleosides previously described by us (*19*, *22*, *23*) and for the measurement of (5′*S*)-8,5′-cdGuo. However, the procedure with the use of succinic acid as the buffer (*21*) emerged more suitable than the other procedures for the measurement of (5′*R*)-8,5′-cdGuo, because the profiles of the typical ions of this compound were obscured due to its early elution from the LC column (see below) when Tris-HCl and phosphate buffers were used. On the other hand, all three procedures were appropriate for the measurement by GC/MS of both (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo. The following procedure was used for all subsequent analyses by LC/MS and GC/MS. DNA samples (100 μg) were dissolved in 100 μL of a 20 mM succinic acid solution (pH 6.0) containing 10 mM $CaCl_2$. Aliquots of (5′*R*)-8,5′-cdGuo-$^{15}N_5$ and (5′*S*)-8,5′-cdGuo-$^{15}N_5$, 5 units of nuclease P1, and 0.04 unit of phosphodiesterase II were added. Samples were incubated at 37 °C for 2 h. Then, 0.003 unit of phosphodiesterase I and 5 units of alkaline phosphatase were added, and the incubation was continued for another 2 h. Subsequently, the samples were filtered using ultrafiltration membranes with a molecular mass cutoff of 5 kDa by centrifugation at 6000*g* for 30 min.

Analyses by LC/MS and GC/MS. LC/MS analyses were performed by using a liquid chromatograph−mass selective detector (1100B Series, Agilent Technologies, Rockville, MD). The atmospheric pressure ionization−electrospray (API-ES) process was employed in the positive ionization mode. The instrument was equipped with a UV spectrophotometer and an automatic sampler. The flow and temperature of the drying gas (nitrogen) were 10 L/min and 350 °C, respectively. The nebulizing gas pressure was 172 kPa. The capillary, fragmentor, and electron multiplier potentials were 4000, 100, and 2600 V, respectively. The quadrupole temperature was 99 °C. Separations were performed using a Zorbax Eclipse XDB C18 reversed-phase column (15 cm × 2.1 mm i.d., 5 μm particle size) (Agilent Technologies, Rockville, MD) with a guard column packed with the same stationary phase (1 cm × 2.1 mm i.d.). Solvent A was a mixture of water and acetonitrile (98/2 v/v), and solvent B was acetonitrile. A gradient of 0.5% of solvent B/min was used. The flow rate was 0.2 mL/min. The column temperature was kept at 30 °C. Aliquots of filtered enzymic hydrolysates of DNA samples were injected on the column without any further treatment. Semipreparative LC was performed using a Supelcosil LC-8 DB reversed-phase column (25 cm × 1 cm i.d., 5 μm particle size) (Supelco, Bellefonte, PA). The solvents and the elution gradient were as above. The flow rate was 2 mL/min. The column was kept at room temperature.

GC/MS measurements were performed using a gas chromatograph (Model 6890 Series)−mass selective detector (Model 5973N) system (Agilent Technologies, Rockville, MD). The column was a fused silica capillary column (12.5 m × 0.2 mm i.d.) coated with cross-linked 5% phenylmethylsilicone gum phase (film thickness, 0.33 μm) (Agilent Technologies, Rockville, MD). Ultrahigh purity helium was used as the carrier gas. The injection port and the GC/MS interface were kept at 250 and 280 °C, respectively. The ion source temperature was 230 °C. The column head pressure was 50 kPa. Filtered enzymic hydrolysates of DNA samples were lyophilized to dryness and then trimethylsilylated with 60 μL of a mixture of bis(trimethylsilyl)-trifluoroacetamide (containing 1% trimethylchlorosilane) and pyridine (1/1 v/v) at 23 °C for 2 h, at 60 °C for 30 min, or at 120 °C for 30 min. Aliquots of 4 μL of the derivatized samples were injected onto the GC column by means of an automatic sampler (Model 7683 series) (Agilent Technologies, Rockville, MD). The split mode of injection with a split ratio of 10 to 1 was used. At this split ratio, which was adjusted electronically, the amount of DNA injected onto the GC column amounted to approximately 0.8 μg. For the measurement of the sensitivity level, a split ratio of 100 to 1 was used. All analyses were performed in the electron ionization mode at 70 eV. The oven temperature of the gas chromatograph was programmed from 130 to 280 °C at a rate of 8 °C/min after 2 min at 130 °C.

RESULTS

Hydroxyl radical-induced 8,5′-cyclopurine 2′-deoxynucleosides are tandem lesions in DNA representing damage to both the sugar moiety and the heterocyclic base of the same purine nucleoside. There is evidence that these lesions might play a role in NER-deficient diseases and contribute to progressive neurodegeneration (*15*, *16*). To gain insight into the biological role of 8,5′-cyclopurine 2′-deoxynucleosides, it is essential to reliably and sensitively identify and quantify these lesions in mammalian DNA. The measurement of these compounds by GC/MS without the isotope dilution technique was previously described (*12*−*14*). We recently reported the measurement of 8,5′-cdAdo by LC/IDMS and also compared it to the measurement by GC/IDMS (*19*). The objective of the present work was to investigate the measurement of 8,5′-cdGuo in mammalian DNA by LC/IDMS. A further objective was to measure this compound by GC/IDMS for comparison.

Separation and Identification of 8,5′-cdGuo by LC/MS and Isolation of Stable Isotope-Labeled Compounds. Authentic material for 8,5′-cdGuo was not available. For this reason, γ-irradiated solutions of dGuo were used to establish liquid chromatographic and mass spectral characteristics of 8,5′-cdGuo. γ-Irradiation of aqueous solutions of dGuo in the absence of oxygen is known to produce both (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo (*13*). Experimental conditions for LC were established by using several types of reversed-phase columns and by analyzing irradiated dGuo samples.

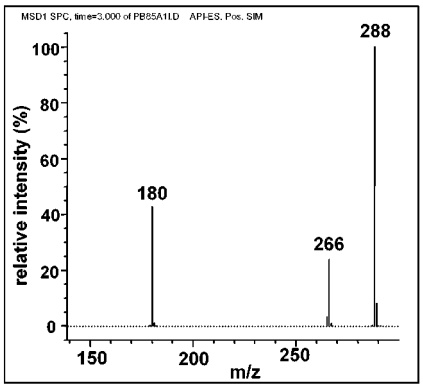

FIGURE 1: API-ES positive ion mass spectrum of (5′*R*)-8,5′-cdGuo. The fragmentor potential was 100 V.

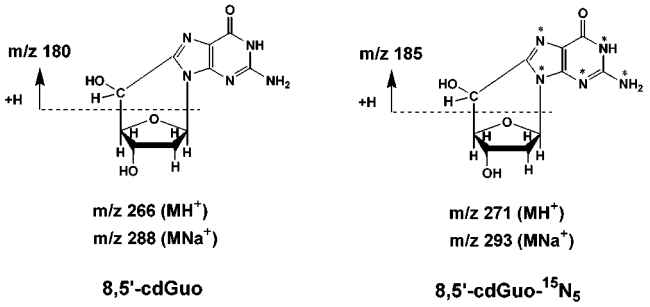

FIGURE 2: Structures and fragmentation pathways of 8,5′-cdGuo and 8,5′-cdGuo-$^{15}N_5$. The stars denote the ^{15}N atoms.

The optimal conditions were obtained by the use of water and acetonitrile as solvents and a 15 cm × 2.1 mm (5 μm particle size) column. Under the experimental conditions used, (5′*R*)-8,5′-cdGuo, (5′*S*)-8,5′-cdGuo, and dGuo eluted at approximately 3.1, 6.7, and 7.6 min, respectively. The other modified nucleosides (5′*R*)-8,5′-cdAdo, 8-OH-dGuo, (5′*S*)-8,5′-cdAdo, and 8-OH-dAdo eluted at 5.2, 9.5, 11.6, and 14.5 min, respectively. We recently reported the measurement of these compounds by LC/MS (*19*, *22*, *23*). Intact nucleosides 2′-deoxycytidine, 2′-deoxythymidine, and 2′-deoxyadenosine had elution times as previously reported (*23*). Mass spectral measurements were performed using the API-ES process in the positive ionization mode. The positive ion mass spectra of (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo were recorded in the total ion monitoring mode. The mass spectra consisted of a fragment ion at *m*/*z* 180, the protonated molecular ion (MH^+) at *m*/*z* 266, and the sodium adduct ion (MNa^+) at *m*/*z* 288. As an example, the mass spectrum of (5′*R*)-8,5′-cdGuo is illustrated in Figure 1. The ion at *m*/*z* 180 is formed by the cleavage of both the glycosidic bond and the bond between the 5′-carbon and 4′-carbon of the sugar moiety with an H atom transfer and contains the base moiety and the 5′-CHOH portion of the sugar moiety plus an H atom (Figure 2). This mechanism is supported by the fact that both 8,5′-cdAdo and 8,5′-cycloadenosine undergo the same fragmentation leading to analogous ions (*19*). (5′*S*)-8,5′-cdGuo yielded a spectrum essentially identical to that in Figure 1 with minor differences in the intensities of the ions, which depended upon the fragmentor potential. At 100 V, MNa^+ constituted the base peak in both spectra with 100% relative intensity. MH^+ had a relative intensity of 25% in both spectra, whereas the *m*/*z* 180 ion had relative intensities of 45% and 70% in the spectra of (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo, respectively. The difference between the relative

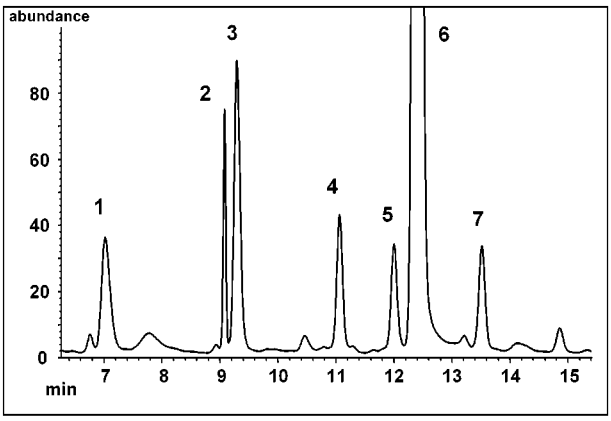

FIGURE 3: Analysis of irradiated and dephosphorylated dGTP-$^{15}N_5$ on a semipreparative LC column with detection by a UV spectrophotometer. The elution conditions are described in Experimental Procedures. Peaks: 1, FapyGua-$^{15}N_5$; 2, (5′*R*)-8,5′-cdGuo-$^{15}N_5$; 3, guanine-$^{15}N_5$; 4, unknown; 5, (5′*S*)-8,5′-cdGuo-$^{15}N_5$; 6, dGuo-$^{15}N_5$; and 7, 8-OH-dGuo-$^{15}N_5$.

intensities of the *m*/*z* 180 ion indicates the stereospecificity of the fragmentation, which is more prominent in the latter diastereomer than in the former. Figure 2 also shows the fragmentation mechanism of 8,5′-cdGuo-$^{15}N_5$, which was used as an internal standard for quantification of 8,5′-cdGuo in DNA by LC/IDMS and GC/IDMS. As expected, the masses of the typical ions of 8,5′-cdGuo-$^{15}N_5$ at *m*/*z* 185, 271 (MH^+), and 293 (MNa^+) were greater by 5 Da than those of 8,5′-cdGuo. The isolation of 8,5′-cdGuo-$^{15}N_5$ is described below.

Next, we attempted to isolate stable isotope-labeled analogues of (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo from irradiated and alkaline phosphatase-hydrolyzed samples of dGTP-$^{15}N_5$ using semipreparative LC. Figure 3 illustrates a chromatogram recorded by the UV spectrophotometer of the LC/MS instrument during the analysis of a sample of irradiated and dephosphorylated dGTP-$^{15}N_5$ using a semipreparative column. Peak 6 represents dGuo-$^{15}N_5$. The compounds represented by peaks 1−5 and 7 were collected. For this purpose, at least 30 injections were made using an aliquot of 100 μL of the dGTP-$^{15}N_5$ sample each time. Collected fractions were dried in a SpeedVac under vacuum. Each dried fraction was dissolved in 200 μL of water. Absorption spectra were taken between the wavelengths of 210 and 350 nm. The compounds represented by peaks 2 and 5 exhibited identical absorption spectra with an absorption maximum at 257 nm. These spectra were identical to the previously published absorption spectra of (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo (*13*) and similar to the absorption spectrum of dGuo (absorption maximum at 252 nm), with the only difference being the shift of the absorption maximum by approximately 5 nm into a longer wavelength. A similar spectral behavior had previously been observed with 8,5′-cAMP when compared with AMP (*4*). Consequently, the compounds represented by peaks 2 and 5 in Figure 1 were assigned to (5′*R*)-8,5′-cdGuo-$^{15}N_5$ and (5′*S*)-8,5′-cdGuo-$^{15}N_5$, respectively. The elution order of these diastereomers had previously been determined using similar LC conditions (*13*). The concentrations of their solutions were 0.0045 and 0.035 mM, respectively, as determined by UV spectroscopy using the absorption coefficient of 13000 M^{-1} cm^{-1} (*24*). The collected compounds were analyzed by

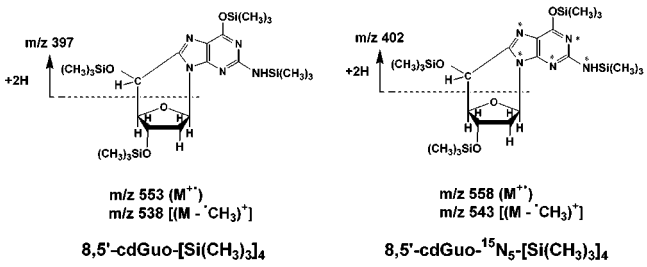

FIGURE 4: Structures and fragmentation pathways of 8,5′-cdGuo-[(Si(CH$_3$)$_3$]$_4$ and 8,5′-cdGuo-$^{15}N_5$-[(Si(CH$_3$)$_3$]$_4$. The stars denote the ^{15}N atoms.

LC/MS using the analytical column to confirm their identity by MS and to check their purity. The mass spectra of (5′*R*)-8,5′-cdGuo-$^{15}N_5$ and (5′*S*)-8,5′-cdGuo-$^{15}N_5$ were similar to those of (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo, respectively, with the exception of the expected shift by 5 Da in the masses of their typical ions as shown in Figure 2. Thus, the LC/MS analysis confirmed the assignment of these compounds by their UV spectra as discussed above.

Isolated compounds were also analyzed by GC/MS after trimethylsilylation. The trimethylsilyl derivatives of (5′*R*)-8,5′-cdGuo-$^{15}N_5$ and (5′*S*)-8,5′-cdGuo-$^{15}N_5$ [(5′*R*)-8,5′-cdGuo-$^{15}N_5$-[(Si(CH$_3$)$_3$]$_4$ and (5′*S*)-8,5′-cdGuo-$^{15}N_5$-[(Si(CH$_3$)$_3$]$_4$, respectively] yielded essentially identical mass spectra with the molecular ion ($M^{+\bullet}$) at *m*/*z* 558 and a typical ($M - {}^{\bullet}CH_3$)$^+$ ion at *m*/*z* 543, which is formed by loss of a methyl radical ($^{\bullet}CH_3$) from $M^{+\bullet}$. Another characteristic ion was observed at *m*/*z* 402, which results from the same type of fragmentation of 8,5′-cdGuo observed when analyzed by LC/MS (Figure 2). The mass spectra of (5′*R*)-8,5′-cdGuo-$^{15}N_5$-[(Si(CH$_3$)$_3$]$_4$ and (5′*S*)-8,5′-cdGuo-$^{15}N_5$-[(Si(CH$_3$)$_3$]$_4$ were essentially identical to the previously published mass spectra of (5′*R*)-8,5′-cdGuo-[(Si(CH$_3$)$_3$]$_4$ and (5′*S*)-8,5′-cdGuo-[(Si(CH$_3$)$_3$]$_4$ (*13*) with the exception of the expected shift by 5 Da in the masses of the ions. The fragmentation of these compounds is illustrated in Figure 4. Other characteristic fragment ions not shown in Figure 4 were observed at *m*/*z* 366, 380, 395, 422, and 448 in the mass spectrum of 8,5′-cdGuo-[(Si(CH$_3$)$_3$]$_4$ as previously reported (*13*) and at *m*/*z* 371, 385, 400, 427, and 453 in the mass spectrum of 8,5′-cdGuo-$^{15}N_5$-[(Si(CH$_3$)$_3$]$_4$.

Peaks 3 and 7 in Figure 3 represent guanine-$^{15}N_5$ and 8-OH-dGuo-$^{15}N_5$, respectively. The measurement of 8-OH-dGuo by LC/MS was previously described (*22*). The compound represented by peak 1 had an absorption spectrum identical to that of 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) with an absorption maximum at 266 nm (*25*). Its mass spectrum was essentially identical to that of FapyGua with typical ions shifted by 5 Da. It consisted of the ions at *m*/*z* 175 (MH^+), 197 (MNa^+), 157, and 147. The last two ions result from the elimination of H_2O (18 Da) and CO (28 Da) from MH^+, respectively. On the basis of its absorption and mass spectra, the compound represented by peak 1 in Figure 3 was assigned to FapyGua-$^{15}N_5$. It might have resulted from the hydrolysis of its nucleoside form 2-amino-4-hydroxy-5-(formylamino)-6-(2′-deoxyribosyl)aminopyrimidine-$^{15}N_5$ during the incubation of irradiated dGTP-$^{15}N_5$ with alkaline phosphatase. The origin of peak 4 in Figure 3 is unknown.

Identification and Quantification of 8,5′-cdGuo in DNA by LC/MS and GC/MS. Using the chromatographic and mass spectrometric characteristics of 8,5′-cGuo, LC/MS in the selected ion monitoring mode (LC/MS−SIM) was used to identify this compound in the enzymic hydrolysates of unirradiated and irradiated DNA samples. The characteristic ions at *m*/*z* 180, 266 (MH^+), and 288 (MNa^+) from the mass spectrum of 8,5′-cdGuo were monitored simultaneously at the appropriate retention time periods, where the (5′*R*)- and (5′*S*)-diastereomers eluted. For quantification, amounts of (5′*R*)-8,5′-cdGuo-$^{15}N_5$ ranging from 0.45 to 4 pmol and (5′*S*)-8,5′-cdGuo-$^{15}N_5$ ranging from 3.5 to 30 pmol were added as internal standards to DNA samples depending on the extent of damage. The characteristic *m*/*z* 185, 271 (MH^+), and 293 (MNa^+) ions of the (5′*R*)-8,5′-cdGuo-$^{15}N_5$ and (5′*S*)-8,5′-cdGuo-$^{15}N_5$ were also recorded during LC/MS−SIM analyses. Figure 5 illustrates the ion current profiles of the molecular ions of 8,5′-cdGuo and 8,5′-cdGuo-$^{15}N_5$ at *m*/*z* 266 and 271, respectively, which were obtained using an enzymic hydrolysate of DNA irradiated at 5 Gy. The ions were recorded by SIM in the narrow time window only, as shown in Figure 5. For this reason, the rest of the chromatogram is not relevant and thus not shown. The results showed the unequivocal identification of (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo in DNA. Another independently prepared set of enzymic hydrolysates of DNA samples was lyophilized, trimethylsilylated, and analyzed by GC/MS−SIM. Characteristic ions of 8,5′-cdGuo-[(Si(CH$_3$)$_3$]$_4$ and 8,5′-cdGuo-$^{15}N_5$-[(Si(CH$_3$)$_3$]$_4$ discussed above were monitored at appropriate retention time periods, where the (5′*R*)- and (5′*S*)-diastereomers of these compounds eluted. The results unequivocally identified both (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo in DNA and also confirmed the identification of these compounds by LC/MS.

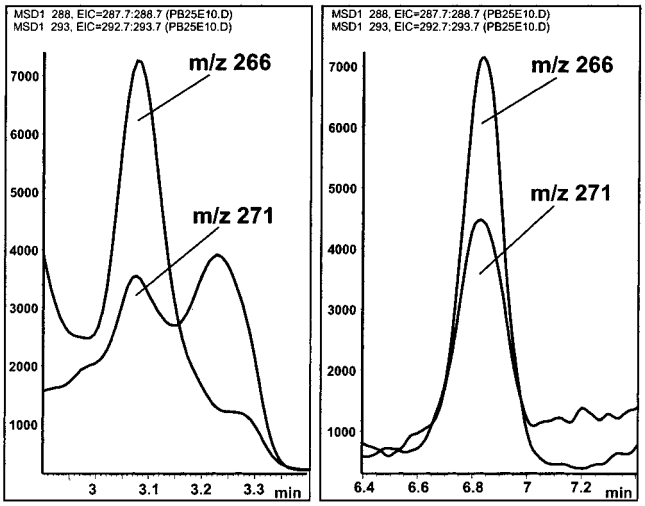

FIGURE 5: Ion current profiles at *m*/*z* 266 (MH^+) of 8,5′-cdGuo and at *m*/*z* 271 (MH^+) of 8,5′-cdGuo-$^{15}N_5$ recorded during the LC/MS−SIM analysis of an enzymic hydrolysate of DNA irradiated at 5 Gy. Peaks: left, (5′*R*)-diastereomers; right, (5′*S*)-diastereomers. The elution conditions are described in Experimental Procedures. The fragmentor potential was 100 V.

The quantification of (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo in unirradiated and irradiated DNA samples was achieved using the integrated areas of the signals of their characteristic ions and those of the internal standards monitored during LC/MS−SIM and GC/MS−SIM analyses. Linear dose−yield plots were obtained in the dose range from 0 to 40 Gy as illustrated in Figure 6. Both techniques yielded

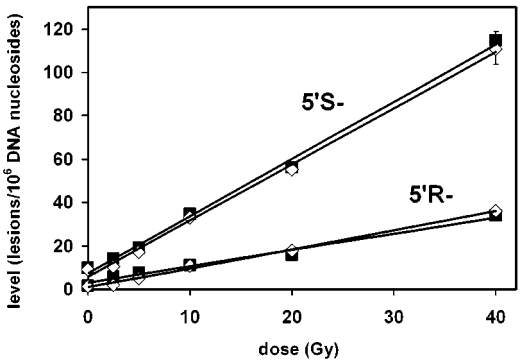

FIGURE 6: Radiation dose−yield plots of 8,5′-cdGuo measured by LC/IDMS and GC/IDMS in DNA exposed to various doses. The data points represent the mean ± standard deviation from the measurement of three independently prepared samples. (■) Measured by GC/IDMS. (◇) Measured by LC/IDMS.

Table 1: Radiation Chemical Yields[a] of 8,5′-cdGuo, 8,5′-cdAdo,[b] and Other Purine-Derived Products[b] in Calf Thymus DNA Exposed to γ-Radiation in N_2O-Saturated Aqueous Solution in the Dose Range from 0 to 40 Gy

	yield [no. of lesions Gy^{-1} (10^6 DNA bases)$^{-1}$]	
lesion	LC/IDMS	GC/IDMS
(5′*S*)-8,5′-cdGuo	2.66 ± 0.05	2.63 ± 0.08
(5′*R*)-8,5′-cdGuo	0.85 ± 0.03	0.74 ± 0.04
8,5′-cdAdo	0.65 ± 0.03	0.70 ± 0.04
8-OH-dGuo[c]	7.77 ± 0.24	8.06 ± 0.17
8-OH-dAdo[c]	2.01 ± 0.03	2.12 ± 0.05
FapyGua		14.30 ± 0.44
4,6-diamino-5-formamidopyrimidine		4.97 ± 0.17
2-hydroxyadenine		0.07 ± 0.03

[a] The yields (±standard deviations) were calculated from the slopes of the linear radiation dose−yield plots by means of a computer program. [b] The yields are from refs *19*, *22*, and *23*. See ref *19* for the yields of pyrimidine-derived products, which were measured by GC/IDMS under identical experimental conditions. [c] Measured by GC/IDMS as 8-hydroxyguanine and 8-hydroxyadenine, respectively.

nearly identical results. The radiation chemical yields of 8,5′-cdGuo were calculated from the slopes of the plots in Figure 6 by means of a computer program. The yields of (5′*S*)-8,5′-cdGuo measured by LC/IDMS and GC/IDMS were 2.66 ± 0.05 and 2.63 ± 0.08 molecules (Gy of radiation)$^{-1}$ (10^6 DNA nucleosides)$^{-1}$, respectively, and those of (5′*R*)-8,5′-cdGuo were 0.85 ± 0.03 and 0.74 ± 0.04 molecules (Gy of radiation)$^{-1}$ (10^6 DNA nucleosides)$^{-1}$, respectively. The ratio of the (5′*S*)-diastereomer to the (5′*R*)-diastereomer was approximately 3.6. The yields of (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo are shown in Table 1 along with the yields of other purine-derived products in DNA, which had previously been measured by LC/IDMS and/or GC/IDMS under similar experimental conditions (*19*, *22*, *23*). The total yield of 8,5′-cdGuo is comparable to the yields of other major purine-derived products 8-hydroxypurines and formamidopyrimidines. Furthermore, it is approximately 4.8-fold greater than that of 8,5′-cdAdo. It should be mentioned that the values shown in Table 1 were obtained under one set of experimental conditions and that experimental conditions, e.g., the presence or absence of oxygen, profoundly affect the yields of DNA modifications (reviewed in refs *2* and *3*). The ratio of (5′*S*)-8,5′-cdGuo to (5′*R*)-8,5′-cdGuo is in contrast to the recently reported ratio of (5′*S*)-8,5′-cdAdo to (5′*R*)-8,5′-

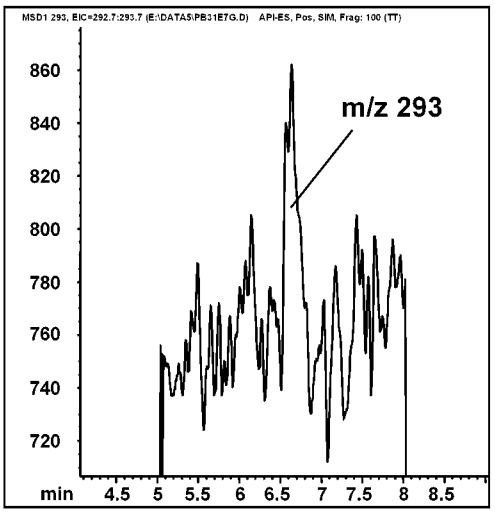

FIGURE 7: Ion current profile at *m*/*z* 293 (MNa^+) recorded during the LC/MS−SIM analysis of a sample of (5′*S*)-8,5′-cdGuo-$^{15}N_5$. The signal corresponds to 15 fmol of (5′*S*)-8,5′-cdGuo-$^{15}N_5$.

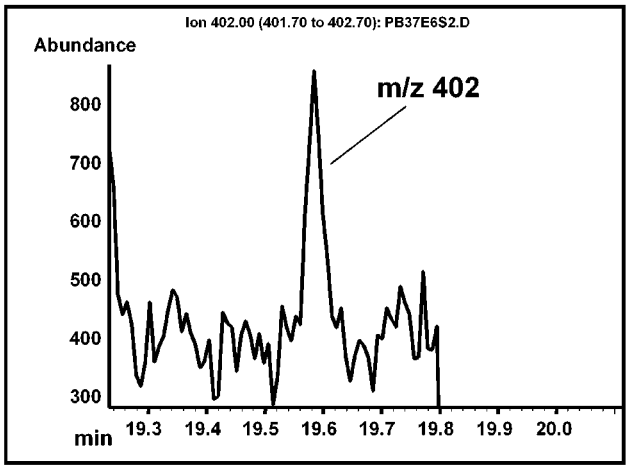

FIGURE 8: Ion current profile at *m*/*z* 402 recorded during the GC/MS−SIM analysis of a sample of (5′*S*)-8,5′-cdGuo-$^{15}N_5$-[(Si-$(CH_3)_3]_4$. The signal corresponds to 1 fmol of (5′*S*)-8,5′-cdGuo.

cdAdo, which was approximately 0.5 (*19*). This means that (5′*S*)-8,5′-cdGuo was formed with an approximately 11-fold greater radiation chemical yield than (5′*S*)-8,5′-cdAdo.

Sensitivity Levels of LC/MS−SIM and GC/MS−SIM for 8,5′-cdGuo. For testing the sensitivity levels of LC/MS−SIM, a dilute solution of (5′*S*)-8,5′-cdGuo-$^{15}N_5$ was analyzed by LC/MS−SIM. Figure 7 illustrates the ion current profile of the *m*/*z* 293 ion (MNa^+). This profile with a signal-to-noise ratio of approximately 3 corresponds to 15 fmol of (5′*S*)-8,5′-cdGuo-$^{15}N_5$ on the LC column. This was the sensitivity level of LC/MS−SIM for this compound. The sensitivity level of GC/MS−SIM for the trimethylsilyl derivative of (5′*S*)-8,5′-cdGuo-$^{15}N_5$ was determined to be 1 fmol. Figure 8 illustrates the ion current profile at *m*/*z* 402, which corresponds to 1 fmol of (5′*S*)-8,5′-cdGuo-$^{15}N_5$-[(Si-$(CH_3)_3]_4$ on the GC column. Samples containing no 8,5′-cdGuo were also analyzed by LC/MS and GC/MS. The results showed no cross-contamination, which might have been a source of the positive results.

Background Levels of 8,5′-cdGuo in DNA of Mammalian Tissues. Background levels of both (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo were measured in commercial calf thymus

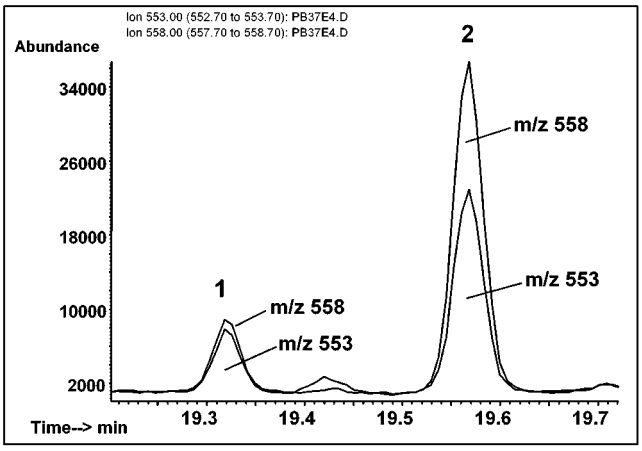

FIGURE 9: Ion current profiles at *m*/*z* 553 ($M^{+\bullet}$) of 8,5′-cdGuo-[$(Si(CH_3)_3]_4$ and at *m*/*z* 558 ($M^{+\bullet}$) of 8,5′-cdGuo-$^{15}N_5$-[$(Si(CH_3)_3]_4$ recorded during the GC/MS−SIM analysis of the trimethylsilylated enzymic hydrolysate of a DNA sample isolated from HeLa cells. Peaks: 1, (5′*R*)-diastereomers; 2, (5′*S*)-diastereomers. GC/MS conditions are described in Experimental Procedures.

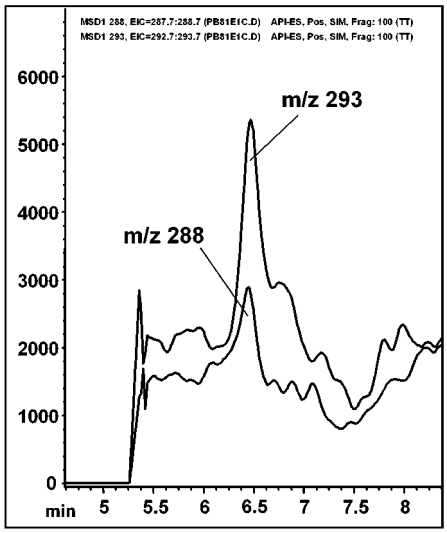

FIGURE 10: Ion current profiles at *m*/*z* 288 (MNa^+) of (5′*R*)-8,5′-cdGuo and at *m*/*z* 293 (MNa^+) of (5′*R*)-8,5′-cdGuo-$^{15}N_5$ recorded during the LC/MS−SIM analysis of the enzymic hydrolysate of an unirradiated calf thymus DNA sample. LC/MS conditions are described in Experimental Procedures.

DNA and in DNA samples isolated from three different types of cultured human cells. In the case of GC/MS, we first investigated the effect of the derivatization temperature because of the possible oxidation of guanine or dGuo during derivatization if improper experimental conditions are used (see, e.g., ref *26*). Derivatization was performed at room temperature for 2 h, at 60 °C for 30 min, or at 120 °C for 30 min. As an example, Figure 9 illustrates the ion current profiles of the molecular ions of 8,5′-cdGuo-[$(Si(CH_3)_3]_4$ and 8,5′-cdGuo-$^{15}N_5$-[$(Si(CH_3)_3]_4$ at *m*/*z* 553 and 558, respectively, which were recorded during the GC/MS−SIM analysis of a DNA sample isolated from HeLa cells. The ions at *m*/*z* 553 and 558 were recorded by SIM in the narrow time window only, as shown in Figure 9. Thus, the rest of the chromatogram is irrelevant and not shown. It was also possible to identify and quantify 8,5′-cdGuo by LC/MS−SIM at background levels in DNA. The ion current profiles at *m*/*z* 288 and 293 illustrated in Figure 10 represent (5′*S*)-8,5′-cdGuo at background level and the internal standard (5′*S*)-8,5′-cdGuo-$^{15}N_5$, respectively, obtained during LC/MS−SIM analysis of an unirradiated calf thymus DNA sample. Figure 11 illustrates the background levels of both (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo in commercial calf thymus DNA and in DNA samples isolated from HeLa, fibroblast, and keratinocyte cells. Both LC/MS and GC/MS provided similar results. In the case of GC/MS, no significant effect of the derivatization temperature was observed on the levels of these compounds. Furthermore, there was no significant difference between the levels of (5′*R*)-8,5′-cdGuo or between the levels of (5′*S*)-8,5′-cdGuo in different cell lines. In all cases, the level of (5′*S*)-8,5′-cdGuo was approximately 5-fold greater than that of (5′*R*)-8,5′-cdGuo.

DISCUSSION

It was previously hypothesized that the cellular repair of 8,5′-cyclopurine 2′-deoxynucleosides might occur via the NER pathway rather than the BER pathway because of the presence of a covalent bond in these molecules (*13*, *14*). Recent studies confirmed this hypothesis and also showed that, if not repaired, these lesions might be cytotoxic, especially in cell lines with NER deficiency (*15*, *16*). Thus far, three techniques were shown to be suitable for the measurement of 8,5′-cyclopurine 2′-deoxynucleosides in DNA. In the 1980s, a methodology using GC/MS was reported for the first time for the detection of both 8,5′-cdGuo and 8,5′-cdAdo in DNA (*12*−*14*). However, the isotope dilution technique was not used for quantification because of the lack of the availability of stable isotope-labeled analogues of these compounds. Recently, both LC/IDMS and GC/IDMS (*19*) and a ^{32}P-postlabeling assay were described for the measurement of 8,5′-cdAdo (*20*), although the latter technique provided no spectroscopic evidence. The results of the present work show that LC/IDMS is also well suited for the detection and quantification of 8,5′-cdGuo. The synthesis and isolation of the stable isotope-labeled analogues of both diastereomers of 8,5′-cdGuo made possible the use of the isotope dilution technique for accurate quantification. The availability of the stable isotope-labeled analogues also facilitated the use of GC/IDMS for this purpose for the first time. Both techniques yielded nearly identical levels of 8,5′-cdGuo in DNA samples, indicating that these two techniques can provide similar results. The sensitivity levels of these techniques for the measurement of 8,5′-cdGuo, however, differed from each other with GC/MS−SIM possessing a substantially greater sensitivity level than LC/MS−SIM. In fact, the difference was approximately 15-fold. Moreover, GC/MS required less DNA amount for analysis than LC/MS. The sensitivity level of LC/MS−SIM for 8,5′-cdGuo (15 fmol) suggests that, if DNA contained 8,5′-cdGuo at a level of 10 molecules/10^6 DNA nucleosides, LC/MS−SIM would facilitate the detection and quantification of this compound using less than 5 μg of DNA. This calculation is based on the equation "1 nmol of a lesion/mg of DNA = 308 lesions/10^6 DNA nucleosides," which is derived from the percent levels of adenine, thymine, guanine, cytosine, and 5-methylcytosine nucleotides in mammalian DNA and their molecular weights (*27*). In the case of GC/MS−SIM, the amount of DNA required for the detection of 10 molecules/10^6 DNA nucleosides would be approximately 0.1 μg. The background level of (5′*S*)-8,5′-cdGuo in mammalian

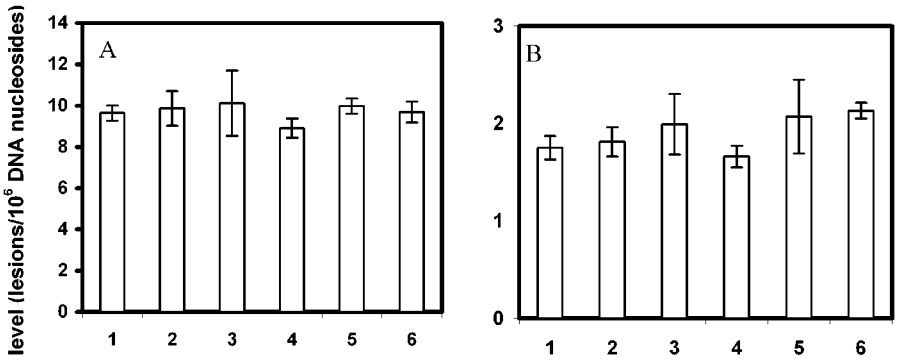

FIGURE 11: Background levels of (5′*S*)-8,5′-cdGuo (A) and (5′*R*)-8,5′-cdGuo (B) in calf thymus DNA and in DNA isolated from cultured cells: 1, calf thymus DNA, derivatization at room temperature ($n = 3$); 2, calf thymus DNA, derivatization at 60 °C ($n = 3$); 3, calf thymus DNA, derivatization at 120 °C ($n = 12$); 4, DNA from HeLa cells, derivatization at 120 °C ($n = 3$); 5, DNA from fibroblast cells, derivatization at 120 °C ($n = 3$); 6, DNA from keratinocyte cells, derivatization at 120 °C ($n = 3$).

DNA was slightly smaller than 10 molecules/10^6 DNA nucleosides. Indeed, approximately 0.3 μg of DNA was used on the GC column to detect this level in mammalian DNA. This amount of DNA also facilitated the detection of (5′*R*)-8,5′-cdGuo at a level of 2 molecules/10^6 DNA nucleosides. The sensitivity level of LC/MS−SIM for 8,5′-cdGuo (15 fmol) is greater than the sensitivity level of this technique for 8-OH-dGuo (35 fmol) (*22*); however, it is lower than that for adenine nucleosides 8,5′-cdAdo (2 fmol) and 8-OH-dAdo (10 fmol). At present, it is not possible to compare the sensitivity level of LC/MS−SIM for 8,5′-cdGuo with that of the other principal mass spectrometric technique LC/MS/MS, because the use of the latter for the measurement of 8,5′-cdGuo was not reported.

Several experimental conditions were applied to check whether an artifactual formation of 8,5′-cdGuo may occur during derivatization of enzymic hydrolysates of DNA samples for GC/MS analysis. There is a potential of artifactual formation of modified nucleosides or bases from intact nucleosides or bases in DNA hydrolysates especially in the case of guanine. It is known that guanine is readily oxidized to give 8-OH-Gua or 8-OH-dGuo, if improper experimental conditions are used for derivatization (see, e.g., ref *26*). However, it is not known whether 8,5′-cdGuo would be formed from dGuo during derivatization. Three different derivatization temperatures were applied. No difference in the level of (5′*R*)-8,5′-cdGuo or (5′*S*)-8,5′-cdGuo was observed. These results suggest that no artifactual formation of 8,5′-cdGuo occurred during derivatization of enzymic hydrolysates of DNA samples under our experimental conditions.

The background levels of 8,5′-cdGuo were similar in DNA isolated from three different human cell lines and in commercially available calf thymus DNA. This might indicate a similar level of oxidative DNA damage and its repair in mammalian cells, at least in terms of this compound. The background level of the (5′*S*)-diastereomer was approximately 5-fold greater than that of the (5′*R*)-diastereomer in calf thymus DNA and in DNA isolated from cultured human cells. As for the radiation chemical yields of these diastereomers in irradiated calf thymus DNA, the (5′*S*)-diastereomer was formed at a greater rate of approximately 3.6 molecules Gy^{-1} (10^6 DNA nucleosides)$^{-1}$ than the (5′*R*)-diastereomer. This is in contrast to 8,5′-cdAdo, the (5′*R*)-diastereomer of which was formed with a approximately 2-fold greater radiation chemical yield than the (5′*S*)-diastereomer under experimental conditions similar to those in the present work (*19*). The reason for this difference between adenine and guanine cyclonucleosides is not known at present. It is expected that the steric hindrances might differently act in both molecules, when the C-5′ centered sugar radical adds to the C-8 position of the purine ring to form the covalent bond between the base and sugar moieties, influencing the preferred formation of one diastereomer over the other one. On the other hand, hydrogen bonding in the double helix might also affect the formation of these compounds, since guanine and adenine possess different hydrogen-bonding properties (*28*). Recent data suggested that the formation of cyclonucleosides in DNA significantly induces a local distortion in the double helix structure of DNA (*17*). The degree of this type of distortion might also depend on the purine involved in cyclization, influencing the diastereomer formation. The differences between the levels of the diastereomers might lead to differences between the biological consequences of these molecules. Thus, it was reported that the predominantly formed (5′*R*)-diastereomer of 8,5′-cdAdo is more efficiently repaired than its (5′*S*)-diastereomer by the human NER system (*16*). The biological properties or the NER-mediated repair of 8,5′-cdGuo was not reported. If the repair properties of 8,5′-cdAdo described above held true for 8,5′-cdGuo, (5′*R*)-8,5′-cdGuo would be expected to be repaired more efficiently than (5′*S*)-8,5′-cdGuo. Since (5′*S*)-8,5′-cdGuo is predominantly formed in contrast to the prevalent formation of (5′*R*)-8,5′-cdAdo, (5′*S*)-8,5′-cdGuo is expected to accumulate in NER-deficient cells more than (5′*S*)-8,5′-cdAdo. Furthermore, the present study showed that the yield of the •OH-induced formation of 8,5′-cdGuo is approximately 4.8-fold greater than that of 8,5′-cdAdo with the ratio of (5′*S*)-8,5′-cdGuo to (5′*S*)-8,5′-cdAdo being approximately 11. Taken together, these facts suggest that the adverse biological effects of 8,5′-cdGuo might significantly outweigh those of 8,5′-cdAdo.

CONCLUSIONS

The results show that LC/MS is well suited for the sensitive and accurate measurement of 8,5′-cdGuo in DNA. The isolation of the stable isotope-labeled analogues of both diastereomers facilitated the use of the isotope dilution technique for quantification. The sensitivity level of LC/MS in the SIM mode amounts to 15 fmol of this compound on

the LC column. At present, it is not possible to compare the measurement of 8,5′-cdGuo by LC/MS with the measurement by the other principal LC/mass spectrometric technique LC/MS/MS, because the use of the latter for this purpose has not been reported. It should be mentioned that the cost of the LC/MS equipment used in this study is approximately 2.5-fold lower than that of an LC/MS/MS equipment. For this reason, LC/MS might be more affordable than LC/MS/MS to use for the measurement of 8,5′-cdGuo or any other modified nucleosides in DNA. Our results show that both (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo can be analyzed by LC/MS simultaneously with other modified nucleosides 8-OH-dGuo, 8-OH-dAdo, (5′*R*)-8,5′-cdAdo, and (5′*S*)-8,5′-cdAdo in the same DNA sample. The use of the GC/MS technique and irradiated DNA samples showed that both LC/MS and GC/MS can yield similar results for the measurement of (5′*R*)-8,5′-cdGuo and (5′*S*)-8,5′-cdGuo, as was previously reported for the measurement of 8-OH-dGuo, 8-OH-dAdo, (5′*R*)-8,5′-cdAdo, and (5′*S*)-8,5′-cdAdo. This paper also reports for the first time that the •OH-induced yield of 8,5′-cdGuo is comparable to those of other principal guanine products 8-OH-Gua (or 8-OH-dGuo) and FapyGua. The greater yield of 8,5′-cdGuo than that of 8,5′-cdAdo and the more abundant formation of the (5′*S*)-diastereomer than the (5′*R*)-diastereomer indicate that the possible adverse biological effects of 8,5′-cdGuo might outweigh those of 8,5′-cdAdo. The methodologies described in this paper might help to gain insight into the possible role of 8,5′-cdGuo in NER-defective diseases in particular and into its biological role in general.

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BI016004D