Analysis and Quantification of DNA Adducts of 2-Amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline in Liver of Rats by Liquid Chromatography/Electrospray Tandem Mass Spectrometry

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Received November 28, 2001

Liquid chromatography with electrospray ionization tandem mass spectrometry (LC/ESI-MS/MS) was used to measure DNA adducts of the carcinogen 2-amino-3,8-dimethylimidazo-[4,5-*f*]quinoxaline (MeIQx) with a microbore C-18 reversed-phase column. Quantification of the isomeric adducts *N*-(deoxyguanosin-8-yl)-2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (dG-C8-MeIQx) and 5-(deoxyguanosin-N^2-yl)-2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (dG-N^2-MeIQx) was achieved using synthetic, isotopically labeled internal standards. The reaction of the *N*-acetoxy ester of 2-(hydroxyamino)-3,8-dimethylimidazo[4,5-*f*]quinoxaline (HONH-MeIQx) with calf thymus DNA (ct DNA) resulted in formation of these adducts in a ratio of 5:1 (dG-C8-MeIQx:dG-N^2-MeIQx). The detection limit by LC/ESI-MS/MS in the selected reaction monitoring (SRM) mode ($[MH^+ \rightarrow MH - 116]^+$) (loss of deoxyribose) approached 500 fg (1 fmol) of adduct standard, and 1 adduct per 10^8 DNA bases using 100 μg of DNA following solid-phase extraction. The SRM analysis of rat liver DNA 24 h after an oral dose of MeIQx (10 and 0.5 mg/kg) revealed the presence of isomeric dG-MeIQx adducts at levels of 3.07 ± 0.84 and 0.45 ± 0.27 adducts per 10^7 bases, respectively. LC/ESI-MS/MS product ion spectra were acquired on both adducts from the elevated dose of MeIQx for unambiguous adduct identification. The contribution of dG-N^2-MeIQx to the total adducts in vivo was significantly more important than that observed in vitro. dG-C8-MeIQx was the principal adduct formed at the 10 mg/kg dose, (dG-C8-MeIQx:dG-N^2-MeIQx (3:2)); however, dG-N^2-MeIQx was the major lesion detected at the 0.5 mg/kg dose (dG-C8-MeIQx:dG-N^2-MeIQx 1:10). The striking differences between the relative amounts of dG-C8-MeIQx and dG-N^2-MeIQx formed in vivo as a function of dose suggest that reactive esters of HONH-MeIQx other than *N*-acetoxy-MeIQx may be formed in vivo and react preferentially with the N^2 atom of guanine, or that dG-C8-MeIQx is removed at a significantly more rapid rate than dG-N^2-MeIQx. The dG-N^2-MeIQx adduct, previously thought to be a minor adduct, is likely to be an important contributor to the genotoxic damage of MeIQx.

Introduction

The heterocyclic aromatic amine (HAA)[1] 2-amino-3,8-dimethylimidazo-[4,5-*f*]quinoxaline (MeIQx, Figure 1) is structurally representative of more than 20 HAAs formed during cooking of proteinaceous foods (*1*, *2*). MeIQx is a potent bacterial mutagen (*3*) and rodent carcinogen, inducing tumors at multiple sites (*4*, *5*). Since MeIQx and other HAAs are consumed daily in a variety of staples, there is concern that HAAs may contribute to human cancer development (*6*).

The adduction of genotoxins to DNA is believed to be the first step in chemically induced carcinogenesis (*7*). Like many other carcinogens, MeIQx must be metabolically activated to bind to DNA and exert its genotoxic effects. Metabolic activation occurs by cytochrome P450 1A2 (P450 1A2)-mediated *N*-oxidation of the exocyclic amine group to form 2-(hydroxyamino)-3,8-dimethylimidazo[4,5-*f*]quinoxaline (HONH-MeIQx) (*8*). This metabolite may undergo phase II conjugation reactions to

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[1] Abbreviations: dG, 2′-deoxyguanosine; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; dG-C8-MeIQx, *N*-(deoxyguanosin-8-yl)-2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; dG-N^2-MeIQx, 5-(deoxyguanosin-N^2-yl)-2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; HONH-MeIQx, 2-(hydroxyamino)-3,8-dimethylimidazo[4,5-*f*]quinoxaline; NO_2-MeIQx, 2-nitro-3,8-dimethylimidazo[4,5-*f*]quinoxaline; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; PhIP, 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine; ct DNA, calf thymus DNA, DTT, dithiothreitol; Me_2SO, dimethyl sulfoxide; HAAs, heterocyclic aromatic amines; I. D., inner diameter; LC/ESI-MS/MS, liquid chromatography electrospray ionization tandem mass spectrometry; MOPS, 3-(*N*-morpholino)propanesulfonic acid; PNK, polynucleotide kinase.

10.1021/tx010178e CCC: $22.00
Published on Web 00/00/0000 PAGE EST: 10.5

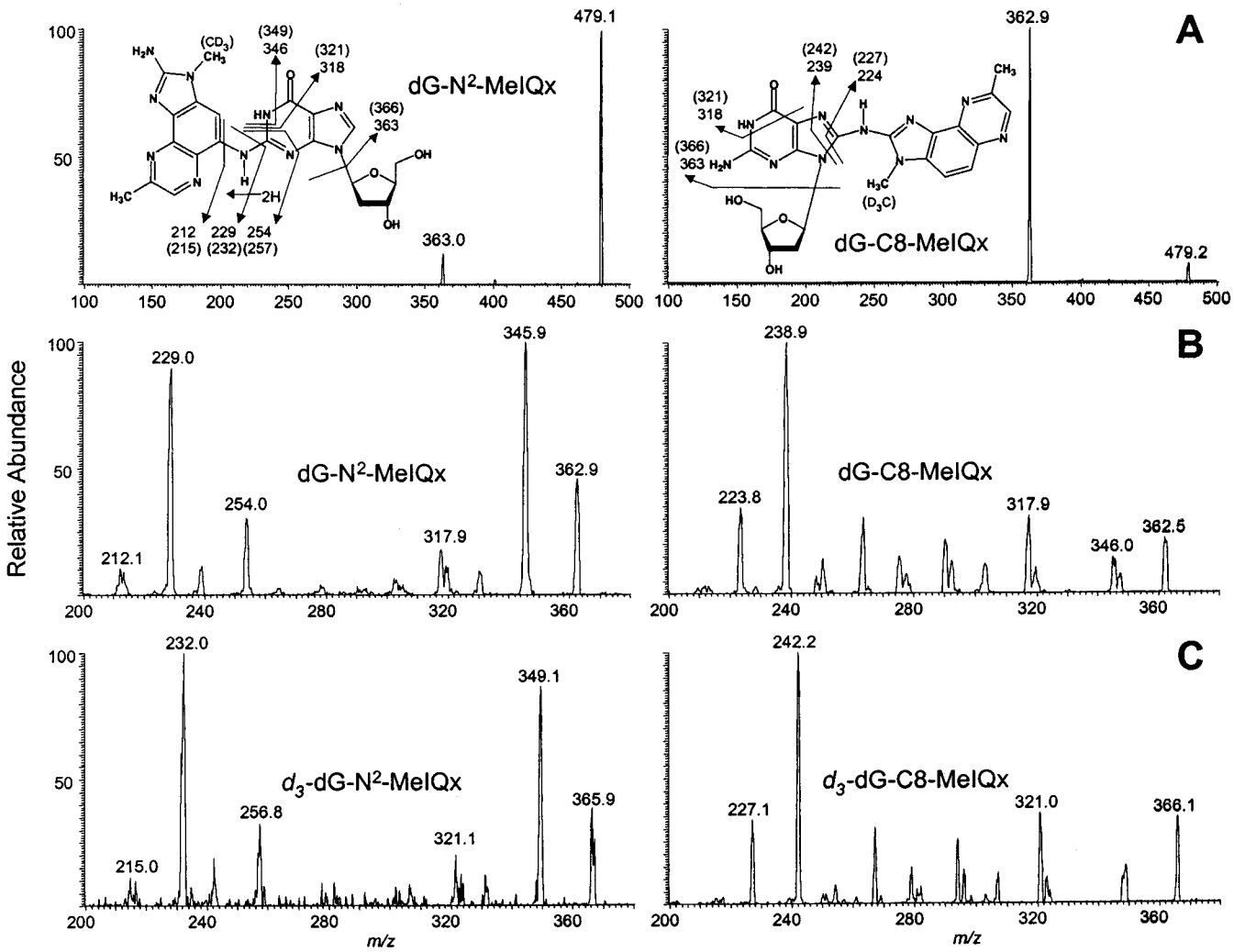

Figure 1. Product ion spectra and proposed mass fragmentation of $m/z = 479$ corresponding to $[M + H]^+$ of dG-N^2-MeIQx (left) and dG-C8-MeIQx (right) at −30 V collision energy (CID offset at −10V, panel A). Panel B: Product ion spectra of $m/z = 363$ (aglycone ion of m/z 479) at −90V collision energy (CID offset at −50V). Panel C: Product ion spectra of $m/z = 366$ (aglycone ion of m/z 482) corresponding to the d_3-labeled internal standards.

form the highly reactive esters, such as *N*-acetoxy or *N*-sulfonyloxy esters, which represent ultimate carcinogenic species (*9*).

DNA adduct formation of aromatic amines and HAAs occurs preferentially at the C-8 atom and to a lesser extent at the N^2 atom of deoxyguanosine (dG) (*10*, *11*); the generation of adducts at the C-8 and N^2 atoms of adenine is usually minor (*10*). In the case of MeIQx, adduction occurs at the C-8 and N^2 atoms of dG to form *N*-(deoxyguanosin-8-yl)-2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline and 5-(deoxyguanosin-N^2-yl)-2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (dG-C8-MeIQx and dG-N^2-MeIQx) (Figure 1) (*12*).

The daily intake of low levels of dietary mutagens and carcinogens may contribute to human cancer risk (*6*, *13*, *14*). Therefore, the identification of exposure and the characterization of biomarkers representing genotoxic damage, such as DNA adducts, may aid in assessing human health risk (*15*, *16*). The ^{32}P-postlabeling assay is currently considered to be the most sensitive technique for the detection of DNA adducts (*17*, *18*), and there have been numerous reports on the detection of DNA adducts of a wide range of genotoxins including HAAs (*11*, *19*−*21*). This assay is commonly used to measure DNA damage because of the high level of sensitivity, where limits of adduct detection can approach 1 adduct per 10^{10} DNA bases (*20*). However, this technique suffers from three major drawbacks: (i) a requirement for high levels of the radioactive isotope ^{32}P, a strong β-emitter and a potential health hazard; (ii) the general lack of suitable internal standards to determine adduct recovery and labeling efficiency; and (iii) the lack of direct structural information on the lesion, which leaves the identity of the adduct ambiguous, particularly in human studies where many adducts may be found (*22*).

Soft ionization techniques such as electrospray ionization (ESI) combined with tandem mass spectrometry have recently emerged as an important technique in the field of DNA adduct analysis (*23*−*26*). On-line liquid chromatography electrospray mass spectrometry (LC/ESI-MS) has been successfully used for the detection and characterization of DNA adducts, where electrospray ionization techniques under micro or nanoflow flow conditions have reached detection limits at the low-femtomole to upper attomole range (*26*, *27*). The aim of this study was to characterize the principal DNA adducts of MeIQx by LC/ESI-MS and to establish a sensitive method for the detection and quantification of MeIQx-derived DNA adducts in vivo as a function of dose.

Materials and Methods

Chemicals and Reagents. *Caution: MeIQx and several of its derivatives are carcinogenic to rodents and should be handled accordingly.*

MeIQx, [2-^{14}C]-MeIQx (specific activity 10 mCi/mmol, radiochemical purity >96%), and [3-$^{2}H_3$C]-MeIQx (MeIQx-d_3, isotopic purity 96.5%) were obtained from Toronto Research Chemicals, [Downsview, Ontario, Canada. Sep-Pak C-18-EC cartridges (Chromabond, 500 mg) were purchased from Machery-Nagel (Düren, Germany]. Acetic acid, acetic anhydride, ascorbic acid, ammonia, Me_2SO, and HPLC solvents (chromatography grade) were from Merck (Darmstadt, Germany). Columns for DNA purification were obtained from Qiagen (Basel, Switzerland). The following chemicals and reagents were obtained from Sigma (Chemical Co., St. Louis, MO): alkaline phosphatase (type III-S), calf thymus DNA (ct DNA), dG, deoxyribonuclease I (type IV), sodium nitrite, TRIS−HCl. Proteinase K, RNase A, and dithiothreitol were obtained from Boehringer Mannheim (Rotkreuz, Switzerland). All other chemicals were reagent grade unless specified otherwise.

Chemical Syntheses of HONH-MeIQx, MeIQx Deoxynucleoside Adducts, and MeIQx-Modified DNA. An isotope dilution of [2-^{14}C]-MeIQx with unlabeled MeIQx was prepared to yield a specific activity of 0.97 nCi/nmol based upon a molar extinction coefficient of 42 000 at 273 nm (in CH_3OH) to serve as a tracer for the exact determination of the molar extinction coefficients of the nucleoside adducts. NO_2-MeIQx was synthesized by the method of Grivas (*28*). The precipitate was washed three times with ice-cold water and redissolved in Me_2SO. The chemical purity exceeded 99% based upon HPLC. The molar extinction coefficient for NO_2-MeIQx was estimated at 17 200 at 305 nm (in CH_3OH). Preparation of HONH-MeIQx was carried out according to Enomoto et al. (*29*) by reduction of NO_2-MeIQx with ascorbic acid except that the reaction was done in Me_2SO (*30*). HONH-MeIQx was purified on a C-18 Sep Pak equilibrated with CH_3OH, followed by ice-cold argon-purged 1 mM EDTA in H_2O. HONH-MeIQx was eluted with 2 mL of argon-purged Me_2SO/C_2H_5OH (4:1, v/v) and immediately used for the synthesis of the deoxynucleoside adducts.

The isomeric dG adducts of MeIQx were prepared according to Turesky et al. (*12*). dG (5 mg/mL) was dissolved in 100 mM sodium phosphate buffer (pH 7.0) containing 1 mM EDTA. The solution (40 mL) was then purged with argon for 10 min and warmed to 37 °C. HONH-MeIQx (0.5 μmol/mL) was then added under argon, immediately followed by a 30-fold molar excess of acetic anhydride. The reaction mixture was quickly capped, mixed, and incubated at 37 °C for 2 h. The brownish precipitate containing mainly adducts was collected by centrifugation and dissolved in a minimum volume of Me_2SO. The adducts were purified directly by preparative HPLC as described below. The adducts remaining in solution were adsorbed onto a C-18 SepPak prewashed with CH_3OH, followed by H_2O. After sample application, the SepPak was washed with H_2O, and the adducts were eluted with CH_3OH and purified by preparative HPLC. The molar extinction coefficients of the isomeric dG-MeIQx adducts were determined based upon a specific activity of 0.97 nCi/nmol to be 50 500 at 305 nm (dG-C8-MeIQx) and 61 000 at 285 nm (dG-N^2-MeIQx). The trideuterated derivatives were prepared in the same manner, but at lower scale (10 mL).

MeIQx-modified calf thymus DNA was prepared by reaction of HONH-MeIQx generated from [2-^{14}C]MeIQx (10 mCi/mmol or 0.1 mCi/mmol) in the presence of acetic anhydride as previously described (*12, 31*). For the optimization of enzymatic digestion conditions, MeIQx-modified DNA at a specific activity of 10 mCi/mmol was used containing 8.9 nmol of MeIQx equivalents per 1 mg of DNA (2.9 adducts per 10^3 unmodified bases). MeIQx-modified DNA at 2.3 nmol of [2-^{14}C]-MeIQx per mg of DNA (0.1 mCi/mmol, 7.6 adducts per 10^4 unmodified bases, 0.5 mg of DNA per mL) was diluted with rat liver DNA from an untreated rat to obtain standard solutions of DNA containing different levels of modification (i.e., 1 adduct per 10^4, 10^5, 10^6, 10^7, and 10^8 unmodified bases).

Animal Treatment and DNA Isolation. Male Fischer-344 rats (220−260 g of body wt, Iffa Credo, L'Abresle, France, n = 4 per group) were acclimated for 1 week and given water and chow ad libitum. MeIQx in 10 mM phosphate-buffered saline (pH 7.4) was administered orally (1 mL) at doses of 0.05, 0.5, and 10 mg/kg body wt using phosphate buffered saline for the control group. The animals were sacrificed 24 h after treatment by anesthesia with pentobarbital (60 mg/kg body wt, i.p.) and after complete removal of blood by the abdominal aorta artery the livers were excised. Liver tissue was homogenized in 3 vol of 10 mM Tris, 140 mM KCl, 10 mM EDTA, and 1 mM DTT (pH 7.4). The nuclear pellet, obtained from 1 g of liver tissue by centrifugation at 10^4 g for 30 min at 4 °C, was diluted with 50 mM MOPS (10 mL) containing SDS (1%, w/v) (pH 7.0) followed by digestion with proteinase K (0.5 mL, 20 mg/mL) for 30 min at 37 °C. Thereafter, RNAse A [4 mL, 100 μg/mL in 50 mM Tris-HCl (pH 8.0)] was added, and the digestion proceeded for 60 min at 37 °C.

The isolation of DNA was done with a Qiagen column according to the protocol provided by the supplier. The DNA concentration was determined by UV absorbance at 260 nm. Typical recoveries were 0.8−1.2 mg of DNA per g of liver tissue. All samples were free (<1%) of RNA contamination as assayed by HPLC after digestion with nuclease P1 and alkaline phosphatase (data not shown).

DNA Digestion and Solid-Phase Purification of Adducts for LC/MS Analyses. Rat liver DNA (250 μg, 0.5 mg/mL) in 10 mM $MgCl_2$/10 mM potassium phosphate (pH 7.0) was digested with DNAse I (30 μg/mL) for 5 h at 37 °C, followed by the addition of 0.012 units/mL phosphodiesterase I and 1.25 units/mL alkaline phosphatase and the digestion proceeded for 18 h. Internal standards (20 pg of dG-C8-MeIQx-d_3 and dG-N^2-MeIQx-d_3 per 100 μg of DNA) were added prior to the digestion. Thereafter, proteins and salt were precipitated with 3 vol of ice-cold C_2H_5OH, followed by centrifugation (5000g, 10 min). The supernatant was retrieved and concentrated to dryness in vacuo. The residue was dissolved with 5 mM K_2HPO_4 acidified to pH 3.5 with CH_3CO_2H (1 mL) and applied to SPE 500 mg C-18 SepPak cartridges for the removal of the major part of the nonmodified deoxynucleosides. The cartridges were preconditioned with CH_3OH (5 mL), followed by 5 mM K_2HPO_4 acidified to pH 3.5 with CH_3CO_2H. After sample loading, nonmodified nucleosides were removed by washing the SepPak with 10% CH_3OH in phosphate buffer (5 mL), followed by H_2O (5 mL). dG-MeIQx adducts were recovered with CH_3OH (3 mL). After evaporation of the eluent, the residue was dissolved in Me_2SO:H_2O (1:1) (25 μL) prior to injection (10 μL) for LC/ESI-MS/MS analysis. Full product ion mass spectra of DNA adducts formed in vivo were acquired on isolated liver DNA (1.5 mg) of a rat treated with MeIQx (10 mg/kg). The DNA was digested to deoxynucleosides and subjected to the sample cleanup procedure as described above. The sample was reconstituted in Me_2SO:H_2O (v/v, 1/1) (15 μL), and 1 mg equivalent of DNA (10 μL) was injected for LC/ESI-MS/MS analysis (for conditions see instrumental section).

Purification and Characterization of DNA Adducts by HPLC with Radioactive Liquid Scintillation Counting. Purification of the deoxynucleoside adducts was done with a Hewlett-Packard 1090M system containing a diode array detector (Geneva, Switzerland). The UV-absorbance was monitored at 264 or 275 nm. A ToshoHaas TK−Gel column (250 × 4.6 mm, Stuttgart, Germany) was used for adduct purification and analyses of enzymatic digestion of calf thymus DNA modified with [2-^{14}C]-MeIQx in vitro. The solvent conditions for the purification of the nucleoside adducts were CH_3CN (5%) in 25 mM $NH_4CH_3CO_2$ (95%) (pH 3.5) initially held for 3 min with a linear gradient to 25% solvent B (CH_3CN) at 25 min, further increased to 100% B at 35 min with a flow rate of 1 mL/min. Fractions of the dG-N^2-MeIQx and dG-C8-MeIQx were collected in 5 mL of 0.5 M $NH_4CH_3CO_2$ (pH 6.8) as a precaution to avoid depurination and lyophilized at 0.02 mbar. The lyophylization was repeated three times after redissolving the residue in

distilled, deionized water (1 mL) to eliminate excess $NH_4CH_3CO_2$. Chromatography in the absence of counterion buffers was difficult due to the strong interactions between the adducts and the column matrix. The $NH_4CH_3CO_2$ minimized peak tailing and significantly improved the chromatography. The HPLC and radioactive analysis of the hydrolyzed calf thymus DNA modified with [2-^{14}C]-MeIQx (10 mCi/mmol) was carried under the same conditions. Radioactive measurements were made by collecting fractions of the HPLC effluent (0.5 mL fractions) into scintillation vials containing counting fluid (10 mL) (UltimaGold, Packard, Meridian, CT) followed by liquid scintillation counting with a LKB 1219 Rackbeta liquid scintillation counter (Perkin-Elmer, Regensdorf, Switzerland).

Characterization of DNA Adducts by LC/ESI-MS/MS. Liquid chromatography electrospray ionization/tandem mass spectrometry (LC/ESI-/MS/MS) was performed with a Hewlett-Packard 1100 HPLC (Geneva, Switzerland) coupled to a Finnigan TSQ 7000 triple quadrupole mass spectrometer (Bremen, Germany) equipped with an API2 interface. Electrospray ionization was set at 3.0 kV ESI-voltage with N_2 used as the sheath gas and set at 80 PSI. The capillary heater was maintained at 350 °C. Data acquisition was performed in SRM mode by monitoring the transition of $[M+H]^+$ $m/z = 479$ (482 for the d_3-internal standards in Q1) to $[MH - 116]^+$ $m/z = 363$ (366) in Q3. The CID offset was set at −10 V for both adducts. The collision energy in Q2 was set to −30 V for the detection of dG-C8-MeIQx and −40 V for dG-N^2-MeIQx. The product ion spectra of the isomeric deoxyguanosinyl-MeIQx adducts were acquired on the corresponding aglycone ions $[MH - 116]^+$, which were generated after cleavage of the deoxyribose linkage prior to Q1 with an elevated CID offset (−50 V). The collision energy in Q2 was set to −40 V. Constant neutral loss scans for the detection of deoxyribose containing molecules were performed with Q2 set to −40 V and a CID offset of −10 V. Argon was used as the collision gas and set at 2.7 mTorr, the scan time was 0.5 s, and the electron multiplier was operated at 1500 V for all analyses.

Chromatographic analysis was done with a 1 × 50 mm C-18 column (GromSil ODS4HE, 3 μm, 1 × 50 mm, Stagroma, Reinach, Switzerland) for adduct separation. A flow rate of 50 μL/min was achieved by splitting the solvent before the autosampler from the original flow rate of 0.46 mL/min by the use of an accurate splitter (LC Packings, Zurich, Switzerland). The gradient was initially 98% A (0.01% CH_3CO_2H) and 2% B (0.01% CH_3CO_2H in CH_3CN) for 2 min, followed by a stepwise increase to 12% B at 2.1 min, which was held at this percent composition until 10 min. Then, there was a stepwise increase to 25% B at 10.1 min, which was held at 25% B until 13 min, followed by a linear increase to 98% B from 13 to 20 min.

Results

Mass Spectrometric Characterization of the Synthetic Nucleoside Adducts. The sites of adduction of the dG-C8-MeIQx and dG-N^2-MeIQx isomers were previously elucidated by ^{1}H NMR (*12*) and now characterized by the electrospray product ion spectra, which are presented in Figure 1. The product ion spectra of the $[M+H]^+$ ions (*m/z* 479) of both adducts revealed similar fragmentation. The major fragment is attributed to the loss of the deoxyribose moiety (116 Da) to give an abundant AH_2^+ ion (*32*, *33*) at *m/z* 363 representing $[MH - 116]^+$. The cleavage of the deoxyribose linkage is more facile for the dG-C8-MeIQx than dG-N^2-substituted derivative under the same collision energy conditions. The relatively facile loss of deoxyribose is a common feature for deoxynucleoside adducts and enables a sensitive and specific detection of covalently modified bases using selected reaction monitoring (SRM) mode or constant neutral loss (CNL) scanning techniques (*33*). Additional structural information was obtained on the corresponding aglycone ions AH_2^+ (*m/z* 363), which were generated after cleavage of the deoxyribose moiety in-source prior to Q1 (Figure 1b). The base peak in the product ion spectrum of dG-N^2-MeIQx was observed at *m/z* 346 and attributed to the loss of NH_3 (17 Da) from the guanine moiety $[AH_2 - NH_3]^+$, following in-source cleavage of the deoxyribose moiety prior to Q1. This fragmentation was less prominent for dG-C8-MeIQx, where there was preferential loss of $[AH_2 - CONH_3]^+$ from guanine to produce the ion at *m/z* 318. Other important fragment ions were observed in the product ion spectrum of dG-N^2-MeIQx at *m/z* 212, 229, and at *m/z* 224 and 229 in the product ion spectrum of dG-C8-MeIQx. These fragment ions are consistent with the respective sites of dG adduction. Identical patterns of fragmentation were observed for the N^3-CD_3-containing molecules, which are shifted by 3 Da, and are consistent with the proposed fragments indicated in Figure 1. Some of these differences in fragmentation of the modifed guanine residues may be used to distinguish between isomeric C8- and N^2-substituted dG adducts as they have been previously also observed for dG-C8- and dG-N^2-substituted adducts of 2-aminofluorene and 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) (*33*, *34*).

Table 1. Stability of dG-N^2-MeIQx and dG-C8-MeIQx (2 pg/μL) as a Function of Solvent for Freshly Prepared Standards

	0.01% CH_3CO_2H in water		50% Me_2SO in water	
time [h]	dG-N^2-MeIQx	dG-C8-MeIQx	dG-N^2-MeIQx	dG-C8-MeIQx
0	100 ± 2[a]	100 ± 4	100 ± 3	100 ± 2
2	95 ± 3	61 ± 8	101 ± 5	98 ± 4
6	75 ± 6	4 ± 3	103 ± 7	97 ± 3
12	60 ± 8	N. D.[b]	98 ± 3	102 ± 4
24	42 ± 7	N. D.	101 ± 4	99 ± 8

[a] Analyses were performed in triplicate. [b] N. D. = not detected.

LC/ESI-MS/MS Analysis of the MeIQx Adduct Standards. The dG-MeIQx adducts interacted strongly with several commonly employed reversed phase C18 resin columns, resulting in poor chromatography and peak tailing. The addition of 0.1−0.5% of HCO_2H or CH_3CO_2H to the solvent system minimized peak tailing; however, the labile deoxyribose moiety underwent cleavage during the solvent vaporization process for LC/MS analysis. The use of a 1-mm I. D. reversed phase HPLC column consisting of a C-18 material with a hydrophilic endcapped material resolved peak asymmetry problems in the presence of 0.01% CH_3CO_2H in the solvent, which also diminished ion suppression. The employment of a stepwise gradient allowed for efficient stacking of the adducts at the column head with the earlier elution of nonmodified deoxynucleosides and less hydrophobic compounds. These chromatographic conditions improved detection limits by narrowing the peak width and resulted in an efficient separation of the two isomeric dG adducts.

During the course of the initial analyses, we noticed a decrease in the response of the dG-MeIQx adducts over time when the adducts were reconstituted in the mobile phase (0.01% CH_3CO_2H in H_2O). This effect is more pronounced for the dG-C8-MeIQx and was partially reversible after ultrasonic sonication of the glass vial used for analysis (data not shown). We therefore presumed that these losses at high dilutions (low picograms/microliter) were due to adsorption of adducts to active sites of the glass injector vials. This effect was completely eliminated by diluting adduct standards in 1:1 Me_2SO:H_2O prior to LC/ESI-MS (Table 1).

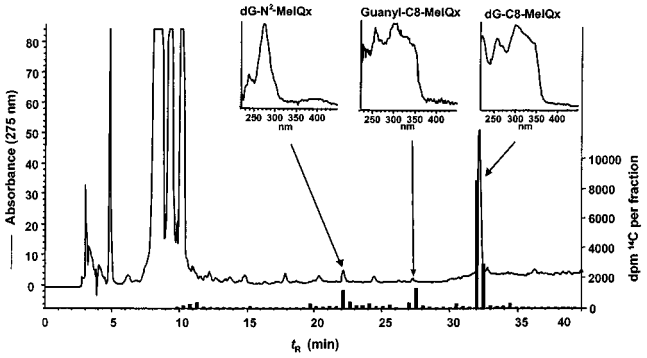

Figure 2. HPLC-profile of 2 mg of digested ct DNA reacted in vitro with [2-^{14}C]-*N*-acetoxy-MeIQx. Inserted UV-spectra and t_R match those of synthetic dG-N^2-MeIQx and dG-C8-MeIQx.

Adduct detection was achieved by monitoring the loss of the deoxyribose moiety in the transition of *m/z* 479 → *m/z* 363 $[MH - 116]^+$ for both isomeric dG-MeIQx adducts. The SRM response was linear in the range from 1 to 500 pg ($r^2 > 0.9975$) with a limit of detection of 750 fg (dG-N^2-MeIQx) and 500 fg (1 fmol dG-C8-MeIQx; S/N 5/1) for the standards. For the quantitative analysis of DNA adducts formed in vivo, a calibration curve for dG-N^2-MeIQx and dG-C8-MeIQx was constructed in the presence of the corresponding N^3-CD_3 internal standards. Increasing amounts of analytes and constant amounts of the respective internal standards were added to liver DNA of an untreated rat and carried through the digestion and extraction steps. A linear dose response was observed for both analytes in the range of 2−100 pg ($r^2 > 0.9859$ and > 0.996 for dG-N^2-MeIQx and dG-C8-MeIQx, respectively). One picogram of each deoxyribonucleoside adduct could be detected with a signal-to-noise ratio of 4/1 and represented the lower limit of detection in digested DNA from rat liver.

MeIQx Adduct Formation in Vitro. CT DNA was modified with HONH-MeIQx in the presence of a 30-fold molar excess of acetic anhydride in the reaction mixture, resulting in high levels of DNA adduct formation (*12*, *31*). The binding of MeIQx to DNA was estimated at 2.9 adducts per 10^3 bases based upon [^{14}C]-content after repeated precipitations with C_2H_5OH and extractions with chloroform/ethyl acetate. This modified DNA was digested to deoxynucleosides and analyzed by HPLC and liquid scintillation counting (Figure 2). Recovery of [^{14}C]-derived radioactivity was >98%, and three principal peaks were detected. One peak with a t_R of 22.5 min accounted for approximately 14.3% of the radioactivity, and the UV spectrum of the product matched that of synthetic dG-N^2-MeIQx with a maximum observed at 285 nm. Another peak at t_R 32.5 min accounted for 60.7% of the radioactivity. The peak displayed a UV absorbance maximum at 305 nm, and the spectrum corresponded to that of dG-C8-MeIQx. These isomeric dG adducts were formed at a ratio of approximately 4:1 (dG-C8-MeIQx: dG-N^2-MeIQx). The radiochromatogram revealed the high efficiency of the enzymatic digestion of the DNA and complete recovery of the dG adducts. Another 10.8% of the radioactivity eluted at t_R 27.5 min, and the product displayed a UV spectrum similar to that of dG-C8-MeIQx. When dG-C8-MeIQx was treated with CF_3CO_2H for 30 min at 80 °C and then analyzed by HPLC, a compound was obtained with an identical t_R and UV spectrum to that of the unknown peak at t_R 27.5 min. This finding suggests that some depurination of dG-C8-MeIQx occurred during the enzymatic hydrolysis of DNA. These enzymatic hydrolysis conditions were employed for all ensuing DNA digestion procedures.

The in vitro modified DNA sample was diluted with unmodified rat liver DNA to yield an adduct concentration of 1 adduct per 10^4 unmodified bases. Enzymatic digests of this modified DNA sample were applied to solid-phase extraction and recovery of adducted deoxynucleosides was estimated to be >99% based upon recovery of radioactivity. An aliquot of digested DNA (100 μg) was analyzed by LC/ESI-MS/MS using the constant neutral loss (CNL) scanning mode for the loss of deoxyribose (loss of 116 Da). The reconstructed ion chromatogram in Figure 3 shows two peaks that are associated with the loss of 116 Da. Both peaks match the t_R of the two synthetic dG-MeIQx adducts and each peak displays an intense $[M + H]^+$ peak at *m/z* 479, with a minor signal attributed to the sodium adduct at *m/z* 501 ($[M + Na]^+$ for both the dG-N^2-MeIQx (t_R 17.6 min) and dG-C8-MeIQx (t_R 20.1 min).

Experiments were carried out on these dG adducts after the cleavage of the deoxyribose moiety to generate the corresponding aglycone ions by increasing the skimmer lens potential to −50 V prior to Q1. The corresponding fragments of *m/z* 363 $[MH - 116]^+$ underwent fragmentation in Q2 at −40 V collision energy to obtain product ion spectra. The total ion current of an aliquot representing 100 μg of DNA containing 1 adduct per 10^4 bases is shown in Figure 4. Two major peaks were detected matching the t_R and product ion spectra of synthetic dG-N^2-MeIQx and dG-C8-MeIQx. A minor peak at t_R 17.5 min could be detected and displayed a product ion spectrum corresponding to guanyl-C8-MeIQx and matched the retention time and product ion spectrum of dG-C8-MeIQx treated with CF_3CO_2H. These findings further support the notion that this third radioactive peak in the HPLC radiochromatogram is guanyl-C8-MeIQx.

The limit of dG-MeIQx adduct detection was determined by serial dilutions of the calf thymus DNA modified with MeIQx at 1 adduct per 10^4 unmodified bases with rat liver DNA from an untreated rat. These serial dilutions were done at levels of modifications containing one adduct in 10^6, 10^7, and 10^8 unmodified bases. Figure 5 depicts the results of the experiments carried out by SRM of the transition of *m/z* 479 → *m/z* 363 for both dG-MeIQx adducts present in DNA digest (100 μg) corresponding to a total of 143 pg (1 adduct per 10^6 bases) of the isomeric adducts (panel A), 14.3 pg (1 adduct per 10^7 bases) (panel B), and 1.4 pg (1 adduct per 10^8 bases) (panel C) assuming 100% digestion efficiency and adduct recovery. The relative responses of the dG-C8-MeIQx:dG-N^2-MeIQx adduct remained constant (4:1) at all levels of adduct modification, indicating that there was no preferential recovery of either adduct. The detection of 1 dG-C8-MeIQx adduct per 10^8 nonmodified bases is well above background levels (10-fold) as demonstrated by the trace of unmodified rat liver DNA (100 μg, panel D) and the trace for 100 μg of unmodified DNA spiked with 20 pg each of dG-C8-MeIQx-d_3 and dG-N^2-MeIQx-d_3 (transition for the nondeuterated compounds, panel E, and transition for their d_3-analogues, panel F). Upon the basis of the data obtained by liquid scintillation counting of digested DNA, this lower limit of quantification corresponds to 0.86 pg of dG-C8-MeIQx using 100 μg of DNA modified in vitro at a level of 1 adduct per 10^8 nonmodi-

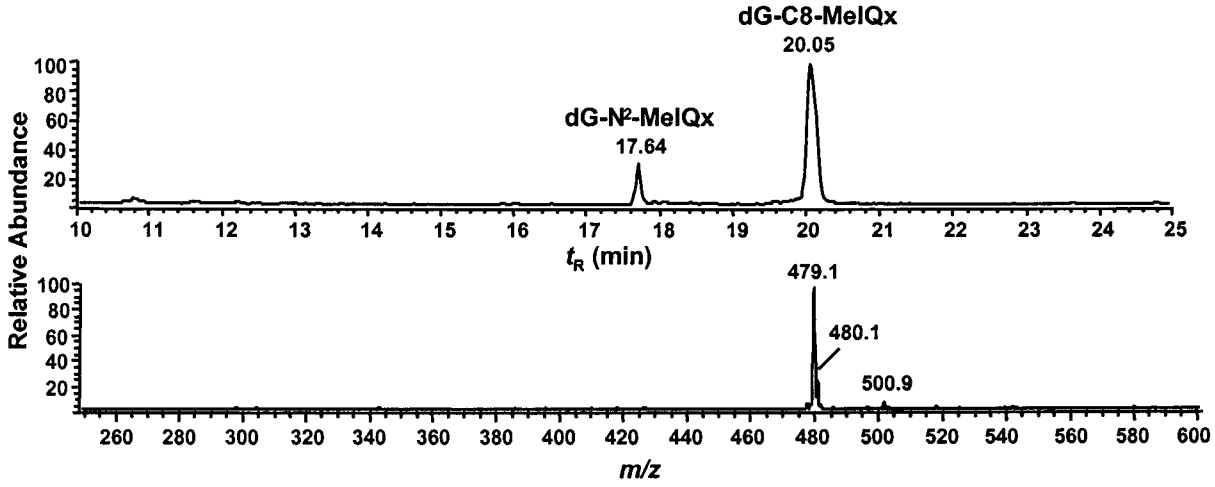

Figure 3. LC/ESI-MS/MS analysis of MeIQx-modified ct DNA in vitro (100 μg of digest of DNA containing 1 adduct/10^4 DNA bases injected) showing dG-N^2-MeIQx (t_R 17.64 min) and dG-C8-MeIQx (t_R 20.05 min) by monitoring the loss of deoxyribose in the constant neutral loss mode with a CID voltage of −10 V and at a collision energy of −40 V in Q2. Both compounds show identical mass spectra with an abundant $[M + H]^+$ ion and a minor signal for the $[M + Na]^+$ ion (lower panel).

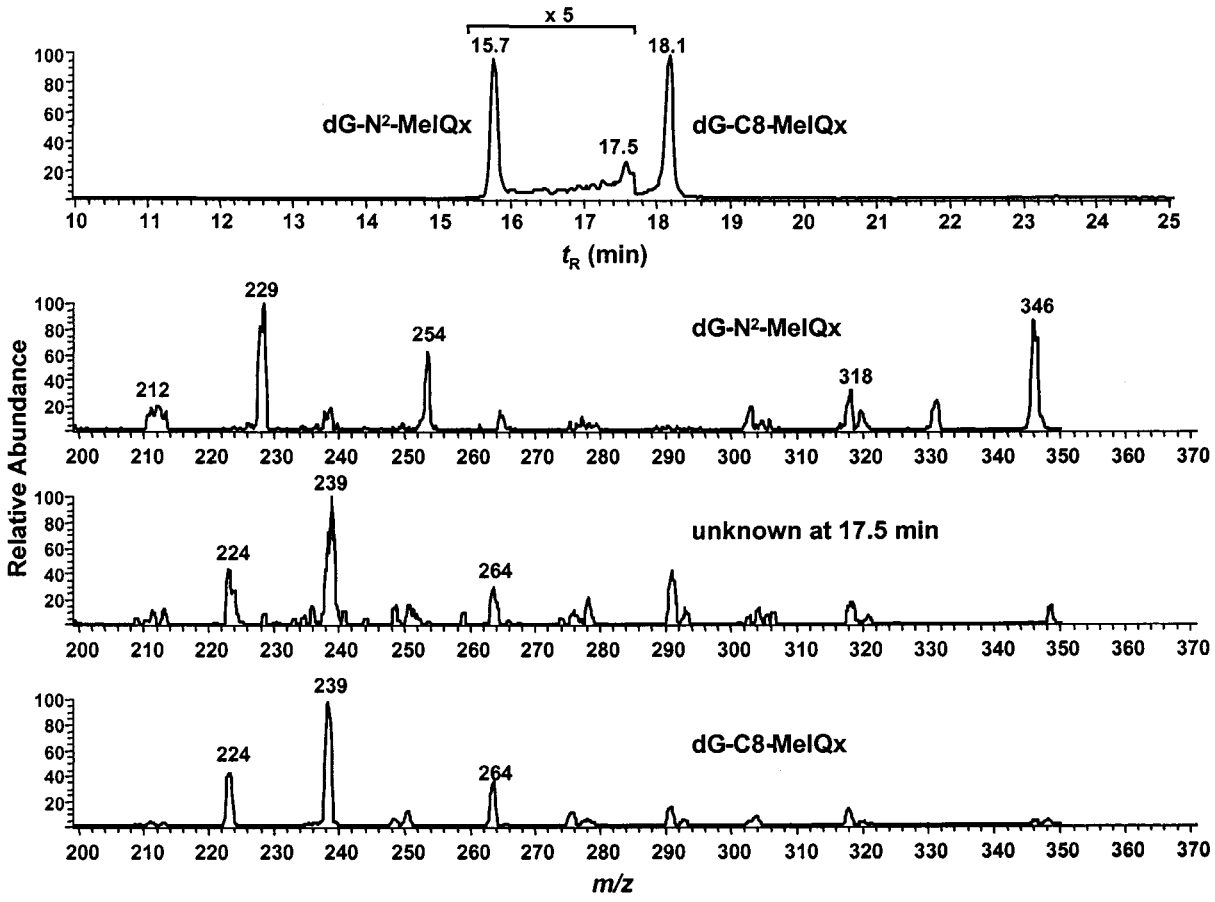

Figure 4. LC/ESI-MS/MS analyses of digested ct DNA modified in vitro with *N*-acetoxy-MeIQx. 100 μg of DNA modified at 1 adduct per 10^4 DNA bases was analyzed monitoring the product ion spectra of $m/z = 363$ (aglycone ion of m/z 479) generated by skimmer lens dissociation at −50 and −40 V collision energy, respectively, for dG-N^2-MeIQx and dG-C8-MeIQx, in Q2.

fied deoxynucleosides. Analyzing 100 μg of the same in vitro modified DNA at the level of 1 adduct per 10^7 unmodified bases, 2 pg of dG-N^2-MeIQx was readily detected. However, other peaks in the ion chromatograms not representing MeIQx-modified DNA bases were present for the transition of DNA adduct standards (m/z 479 → m/z 363). These peaks eluted before dG-C8-MeIQx and in the ion chromatograms of the deuterated internal standards (m/z 482 → m/z 366) and became more important at lower levels of DNA modification. These interferences originate from the enzyme preparations used for DNA digestion. We were unable to remove these interfering components by enzyme purification (repeated precipitation with $(NH_4)_2SO_4$) or different solid-phase extraction procedures used for the enrichment of modified deoxynucleosides (A.P. and R.J.T., unpublished observations). Analyses of DNA samples containing 1 adduct in 10^6, 10^7, and 10^8 normal bases resulted in a linear "dose"

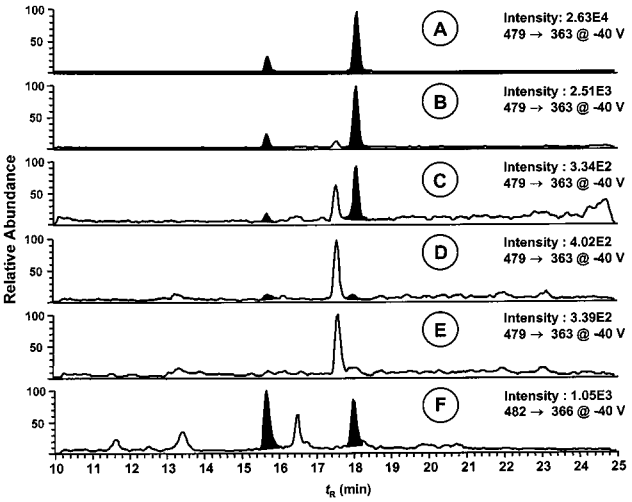

Figure 5. LC/ESI-MS/MS analyses of digested ct DNA reacted in vitro with *N*-acetoxy-MeIQx. Adducted calf thymus DNA was diluted with rat liver DNA from an untreated rat to yield DNA containing decreasing number of adducts: 100 μg of digested DNA were injected for analysis after SPE-enrichment at the level of 1 adduct per 10^6 bases (A), 1 adduct per 10^7 bases (B), 1 adduct per 10^8 bases (C), control DNA from rat liver (D), and trace for 20 pg of the internal standards dG-N^2-MeIQx-d_3 and dG-C8-MeIQx-d_3 (F) in control DNA from rat liver (E).

Table 2. Area Response for the Isomeric Adducts dG-N^2-MeIQx and dG-C8-MeIQx in ct DNA Modified in Vitro with HONH-MeIQx in the Presence of Acetic Anhydride, Followed by Serial Dilution with Unmodified Rat Liver DNA to Different Levels of Modification

adduct level	peak area dG-N^2-MeIQx	peak area dG-C8-MeIQx
1 per 10^5	$(6.60 \pm 0.93)\ 10^5$ [a]	$(2.28 \pm 0.13)\ 10^6$
1 per 10^6	$(6.61 \pm 0.85)\ 10^4$	$(2.51 \pm 0.08)\ 10^5$
1 per 10^7	$(5.29 \pm 0.61)\ 10^3$	$(2.32 \pm 0.28)\ 10^4$
1 per 10^8	$(5.19 \pm 0.82)\ 10^2$	$(2.61 \pm 1.5)\ 10^3$
linearity (R^2)	0.9998	0.9999

[a] Analyses were performed in triplicate using 100 μg of digested DNA.

response, indicating the usefulness of this approach for the quantitative analysis of DNA adducts by this method (Table 2).

MeIQx Adduct Formation in Vivo. Male Fischer F-344 rats were dosed with MeIQx (single oral dose of 10, 0.5, or 0.05 mg/kg, $N = 4$) and sacrificed after 24 h. The two isomeric dG-MeIQx adducts in DNA liver samples were analyzed by LC/ESI-MS/MS in the SRM mode in the presence of the respective deuterated internal standards to compensate for any losses occurred during enzymatic digestion and solid-phase extraction procedures. The results are summarized in Table 3. Two independent sample preparations of DNA per treatment group were carried out and analyzed in duplicate. Figure 6 shows the four ion chromatograms of the respective analytes and trideuterated internal standards of a DNA sample (100 μg digest injected on-column) from a rat treated with MeIQx (10 mg/kg). A total of 3.06 ± 0.83 adducts in 10^7 unmodified bases was detected (both isomeric MeIQx adducts). dG-N^2-MeIQx accounted for 1.28 ± 0.38 adducts per 10^7 unmodified bases (range 0.84−1.73) and dG-C8-MeIQx accounted for 1.78 ± 0.47 per 10^7 unmodified bases (range 1.12−2.13).

SRM analyses also showed the presence of these dG adducts at the intermediate dose (0.5 mg/kg). However, the ratio of the dG-C8-MeIQx:dG-N^2-MeIQx (approximately 3:2 at 10 mg/kg MeIQx) was inverted at this intermediate dose (Figure 7). Total adducts were estimated at 0.45 ± 0.27 adducts per 10^7 unmodified bases with dG-N^2-MeIQx accounting for 0.40 ± 0.26 (range 0.22−0.78) and dG-C8-MeIQx for 0.04 ± 0.06 adducts per 10^7 unmodified bases (range 0.00−0.11). The isomeric dG-MeIQx adduct internal standards were also readily detected by SRM analyses at the lowest dose (0.05 mg/kg); however, MeIQx-DNA adduct formation was below the limit of detection (data not shown).

Table 3. dG-N^2-MeIQx and dG-C8-MeIQx Adduct Formation in Liver DNA of Male Fischer-344 Rats 24 h after a Single Oral Dose

MeIQx [mg/kg]	dG-N^2-MeIQx [adducts/ 10^7 bases]	dG-C8-MeIQx [adducts/ 10^7 bases]	total [adducts/ 10^7 bases]
10	1.28 ± 0.38[a] (0.84−1.73)[b]	1.78 ± 0.47 (1.12−2.13)	3.06 ± 0.83
0.5	0.40 ± 0.26 (0.22−0.78)	0.04 ± 0.06 (0.00−0.11)	0.45 ± 0.27
0.05	N. D.[c]	N. D.	N. D.

[a] $n = 16$ (4 animals per treatment group, 2 independent samples per animal, 2 analyses per sample). [b] Observed range. [c] N. D. means not significantly different from untreated control when 100 μg of digested DNA was analyzed.

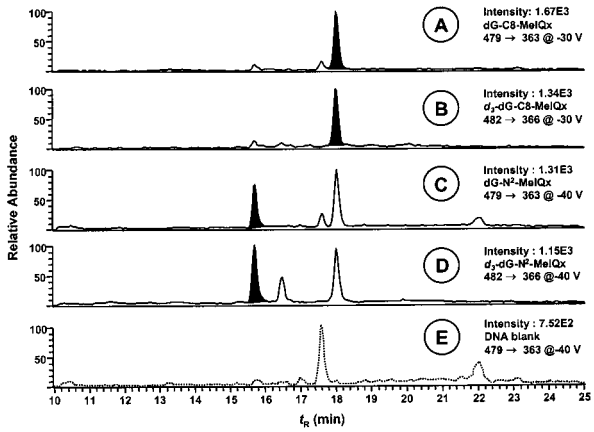

Figure 6. LC/ESI-MS/MS analysis of rat liver DNA (100 μg) 24 h after a single dose of 10 mg/kg MeIQx. The transitions in MRM mode represent dG-C8-MeIQx, dG-C8-MeIQx-d_3, dG-N^2-MeIQx, and dG-N^2-MeIQx-d_3 (from top to bottom). The collision energy in Q2 was set to −30 V for the detection of dG-C8-MeIQx and −40 V for dG-N^2-MeIQx.

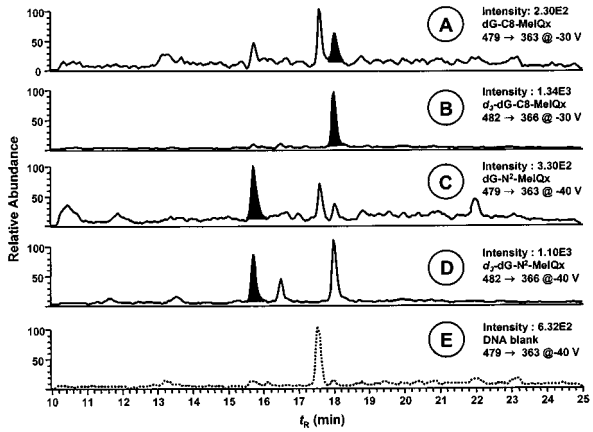

Figure 7. LC/ESI-MS/MS analysis of rat liver DNA (100 μg) 24 h after a single dose of 500 μg/kg MeIQx. The transitions in MRM mode represent dG-C8-MeIQx, dG-C8-MeIQx-d_3, dG-N^2-MeIQx, and dG-N^2-MeIQx-d_3 (from top to bottom). The collision energy in Q2 was set to −30 V for the detection of dG-C8-MeIQx and −40 V for dG-N^2-MeIQx.

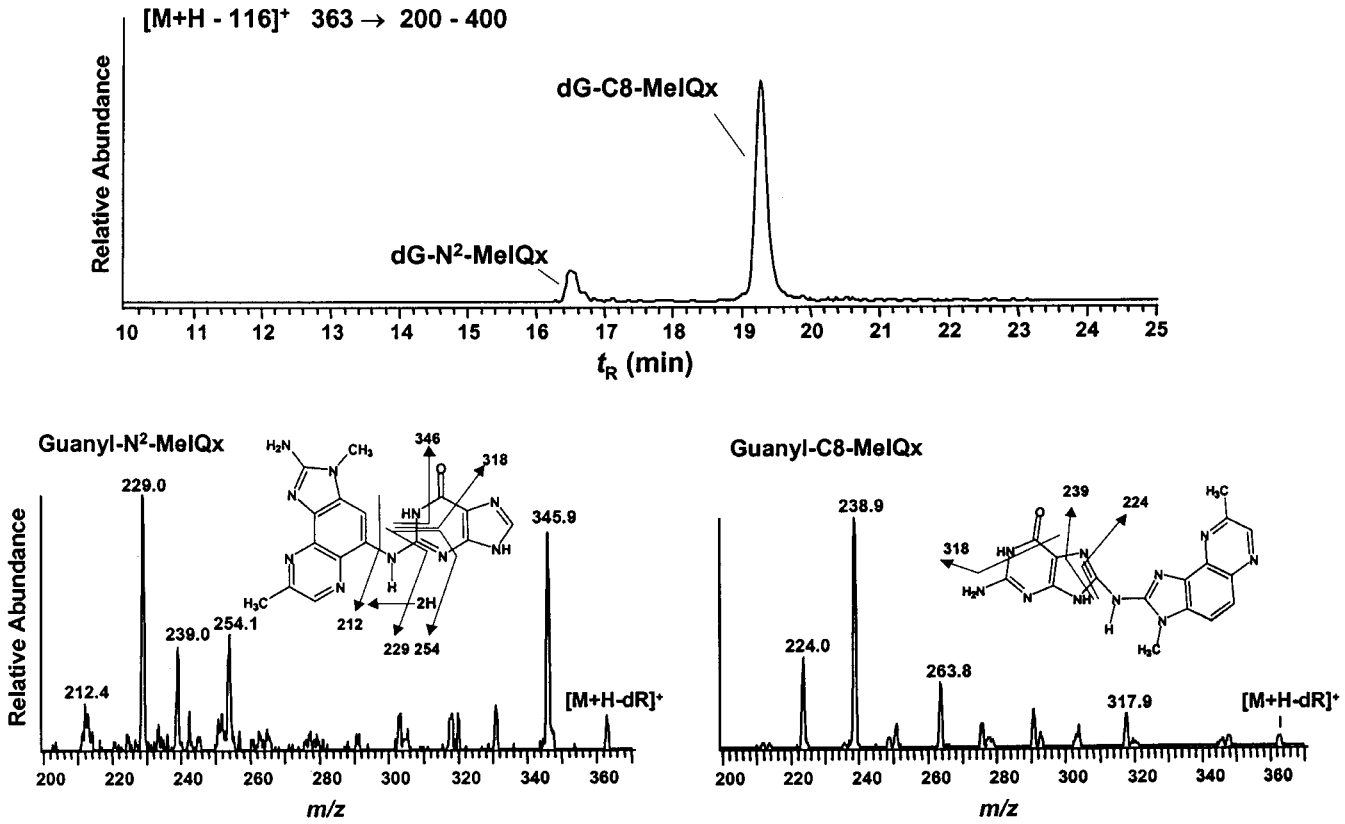

Figure 8. LC/ESI-MS/MS analysis of rat liver DNA (1 mg) 24 h after a single dose of 10 mg/kg MeIQx. Acquisition was made in the product ion mode of the aglycone ion (*m*/*z* 363), which was generated by in source dissociation at −50 V of the parent deoxynucleosides. Product ion spectra were obtained with a collision energy of −40 V in Q2 and match those of synthetic dG-N^2-MeIQx, and dG-C8-MeIQx.

The identities of these isomeric dG-MeIQx adducts in liver were confirmed unambiguously by acquisition of their respective product ion spectra following a large scale work up of the DNA (1.5 mg) from a rat treated with MeIQx at 10 mg/kg. Following solid-phase extraction, the product ion spectra were obtained on the aglycone ions of the dG adducts *m*/*z* 363 $[MH - 116]^+$, which were generated in source prior to Q1. Figure 8 depicts the reconstructed ion chromatogram showing relatively broad peaks due to the large amount of digested DNA loaded onto the HPLC column. Nevertheless, product ion spectra were readily obtained on both isomeric dG-MeIQx adducts and match those of the synthetic adduct standards, thus providing unequivocal confirmation as to their identities.

Discussion

Previous studies utilizing the ^{32}P-postlabeling technique have suggested that MeIQx forms numerous DNA adducts; the multiplicity of lesions was probably attributed to incompletely digested oligomeric adducts (*35*, *36*). More recent ^{32}P-postlabeling studies have revealed the presence of only two or three major spots (*37*, *38*) and dG-C8-MeIQx was identified as a major lesion (*38*). The dG-N^2-MeIQx reference standard was not available in these studies and consequently not identified. We previously reported that the dG-N^2 adduct of the structurally related analogue IQ was a poor substrate for polynucleotide kinase (PNK) and not efficiently labeled in a DNA digest unless an adduct enrichment procedure was performed to remove excess normal nucleotides prior to labeling (*39*). Given the structural similarities in the dG-N^2 adducts of IQ and MeIQx (*12*), dG-N^2-MeIQx also may be a poor substrate for PNK, and may have been overlooked in these postlabeling studies.

Both isomeric dG-MeIQx adducts were identified in liver of rats following a single dose of MeIQx by LC/ESI-MS/MS using SRM detection. Moreover, the identities of both adducts were corroborated by the product ion spectra of the corresponding aglycone derivatives and provided a number of unique, characteristic fragment ions, which were in excellent agreement to those of the reference standards for unequivocal confirmation of their identities. The ability to detect and characterize DNA adducts by LC/ESI-MS/MS product ion spectra is an important advancement that has enabled us to unambiguously confirm the identity of dG-MeIQx adducts in vivo at adduct levels of 1 adduct per 10^7 DNA bases. To the best of our knowledge, this is the first time that complete product ion spectra of DNA adducts have been obtained from in vivo samples with a triple stage quadrupole mass spectrometer.

Quantitative differences were observed between the relative amounts of isomeric dG-MeIQx adducts formed in vitro and in vivo as a function of dose. dG-C8-MeIQx was the predominant adduct formed in vitro with ct DNA modified with the putative carcinogenic metabolite *N*-acetoxy-MeIQx, where the ratio of these dG adducts was estimated at 5:1 (dG-C8-MeIQx (including C8-Guanyl-MeIQx):dG-N^2-MeIQx). The analysis of adducts in rat liver DNA revealed that the contribution of dG-N^2-MeIQx to the total adducts was relatively more important than that observed in vitro. The dG-MeIQx adduct formation in rat liver 24 h following treatment with MeIQx at an

elevated dose (10 mg/kg) occurred in a ratio of 3:2 (dG-C8-MeIQx:dG-N^2-MeIQx). However, the dG-N^2-MeIQx adduct predominated at the intermediate administered dose (500 μg/kg) and was the major adduct present in rat liver DNA 24 h posttreatment (dG-C8-MeIQx:dG-N^2-MeIQx, 1:10). We were unable to measure dG-MeIQx adducts at the lowest dose treatment (0.05 mg/kg), where adduct formation was below the limit of detection (approximately 0.5−1 adduct per 10^8 bases).

Methodological issues on enzymatic digestion and isolation of DNA adducts can be excluded as a basis for this striking difference in isomeric MeIQx-DNA adduct formation in vivo as a function of dose (Table 2, Figure 5). There was no preferential recovery of dG-N^2-MeIQx over dG-C8-MeIQx during the DNA hydrolysis or solid phase adduct enrichment at different levels of adduct modification. The recovery of both adducts remained constant when ct-DNA, which was modified predominantly with dG-C8-MeIQx, was diluted with unmodified DNA from rat liver to levels of adduct modification observed in vivo (vide supra). Errors associated with quantitative analysis also may be excluded as both deuterated adducts, which were used as internal standards and added to DNA prior to the digestion procedure, were recovered with comparable efficiencies. The inversion of dG-C8-MeIQx and dG-N^2-MeIQx adduct formation in vivo as a function of dose treatment, suggests that the relative amounts of dG-C8-MeIQx and dG-N^2-MeIQx formed by reaction of *N*-acetoxy-MeIQx with DNA may be concentration dependent; alternatively, reactive esters of HONH-MeIQx, other than *N*-acetoxy-MeIQx (*8*, *9*), may be formed in vivo and react preferentially with the N^2 atom of guanine.

The relative stablities of dG-C8-MeIQx and dG-N^2-MeIQx in vivo are not known. DNA adducts of MeIQx were reported to be rapidly removed from rat liver tissue based upon ^{32}P-postlabeling and accelerator mass spectrometry (AMS); however, the rates of removal of these isomeric dG adducts were not determined (*36*, *40*). In the case of the structurally related analogue 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), the dG-C8-IQ adduct was removed more rapidly than the isomeric dG-N^2-IQ from liver and kidney of rats, but there was no evidence of selective removal of either dG-C8-IQ or dG-N^2-IQ in colorectum tissue, where removal of both adducts closely followed cell turnover (*41*). Moreover, dG-C8-IQ was found to be the predominant lesion in nonhuman primates treated with a single dose of IQ when measured by ^{32}P-postlabeling. However, dG-N^2-IQ accumulated during chronic treatment and became the predominant lesion in all slowly dividing tissues, indicating preferential removal of the dG-C8-IQ adduct (*42*). These trends in adduct formation and persistence were subsequently corroborated by LC-MS (34).

Structural characterization of the dG-C8 and dG-N^2 adducts of IQ and MeIQx by ^{1}H NMR (*12*) revealed that the dG-C8 adducts exist preferentially in the syn form, which may destabilize the DNA helix (*43*), while the dG-N^2-isomers preferentially occur in the anti form, as does dG. Thus, the differences in rates of removal of isomeric dG-IQ and dG-MeIQx adducts may be due to the differences in DNA adduct conformation, which influence adduct recognition and removal by enzyme repair systems (*43*, *44*). A recent study on the structurally related HAA 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) has reported that the dG-C8 adduct of PhIP in an 11-mer duplex may exist in at least two different conformations (*45*), which may influence the persistence and biological effects of the lesion. Comparable studies on elucidation of the structures and conformations of the isomeric dG adducts of MeIQx and IQ in DNA also may help to explain some of the differences in persistence of these isomeric adducts and their recognition by repair enzymes.

There are two reports on the detection of MeIQx-DNA adducts in humans. Totsuka and co-workers (*46*) employed the ^{32}P-postlabeling technique to identify dG-C8-MeIQx at levels between 1.8 and 18 adducts per 10^{10} unmodified bases in colon and kidney tissue. The dG-N^2-MeIQx adduct was not observed, but as stated above, no reference standard was available and this adduct may have been overlooked. AMS has also been used for the measurement of MeIQx-derived DNA adducts at low doses in human colon, where HPLC and offline AMS measurements of human subjects given 21.3 μg of [^{14}C-MeIQx] provided evidence for the formation of MeIQx-DNA adducts in a ratio of approximately 2:1 (dG-C8-MeIQx:dG-N^2-MeIQx) 3.5−6 h post-treatment (*47*).

Since DNA adduct formation is generally considered to be the first step in chemically induced carcinogenesis (*7*, *48*), methods to quantify DNA adducts for risk assessment are essential. A relationship between DNA adduct formation and cancer development in humans has only been established for aflatoxin B_1 (*49*, *50*), in part because of the difficulty to quantitatively measure DNA adducts at low levels of adduct modification with limiting amounts of DNA. Both the ^{32}P-postlabeling technique and more recently AMS have been used to screen for DNA adduct formation, where the limits of detection have been reported to approach 1 adduct in 10^{10} (*17*, *18*), and 1 adduct in 10^{15} unmodified bases, respectively (*47*). The sensitivity of both methods still surpass the sensitivity of LC/ESI-MS/MS; however, both techniques lack structural data for the unambiguous identification of the adducts, which LC/ESI-MS methods provide.

LC/ESI-MS/MS detection of MeIQx-derived DNA adducts was achieved with a limit of quantitation of about 1 adduct per 10^8 unmodified DNA bases using 100 μg of DNA. This level of sensitivity was achieved with a 1-mm i.d. C-18 column at a flow rate of 50 μL/min. Further improvements on the detection limits may be achieved by capillary liquid chromatography/microelectrospray/mass spectrometry (*27*). However, there are several advantages of employing a 1-mm I. D. column over a capillary column; these include larger injection volumes, more robust columns with superior loading capacity, and prolonged lifetime as compared to the capillary column system. Nevertheless, Soglia et al. (*27*) and Gangl at al. (*51*) have demonstrated that a microLC system coupled to ESI-MS/MS could achieve high levels of sensitivity, where detection limits of isomeric IQ-DNA adducts of nonhuman primates and rats approached 2 adducts per 10^8 bases using 300 μg of DNA even though only a small portion of the sample was injected on the column. Further studies are required to evaluate the robustness of the capillary LC techniques as opposed to microcolumn chromatography to determine which method is most applicable for large scale population biomonitoring studies.

Several other studies have been reported on aromatic amine and HAA−DNA adduct detection by LC/ESI-MS. The detection of the C8-deoxyguanosinyl adduct of 4-

aminobiphenyl (ABP) in hepatic DNA of mice treated with ABP was reported (*24*), where detection limits of 7 ABP adducts in 10^8 unmodified bases was achieved using 100 μg of DNA. For another HAA, PhIP, a limit of quantitation for the major dG-C8 adduct in PhIP-modified ct-DNA was estimated at 2.7 adduct per 10^7 using 500 μg of DNA in the multiple ion monitoring mode (*52*).

In summary, a specific and sensitive LC/ESI-MS/MS method has been established for the detection and quantification of MeIQx-derived DNA adducts employing stable isotopically labeled internal standards. Both dG-N^2-MeIQx and dG-C8-MeIQx were the only DNA adducts detected in vitro or in vivo. This methodology enables the detection of approximately 1 adduct per 10^8 unmodified bases using 100 μg of DNA. Future improvements on ionization efficiency and interface technology may further improve the limits of detection and permit the routine application of LC/ESI-MS/MS in assaying for dG-MeIQx adducts in human biomonitoring studies.

Acknowledgment. This work was supported in part by a grant from the National Institutes of Health (1RO1CA69390-06) (J.S. and P.V.). The comments of Dr. F. Beland and Dr. F. Kadlubar, NCTR, are greatly appreciated.

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