SHORT COMMUNICATION

Characterization of an N^6-oxopropenyl-2'-deoxyadenosine adduct in malondialdehyde-modified DNA using liquid chromatography/electrospray ionization tandem mass spectrometry

Ajai K Chaudhary^1,2,3, G Ramachandra Reddy^1,2, Ian A Blair^3 and Lawrence J Marnett^1,2,4

^1A B Hancock Jr Memorial Laboratory for Cancer Research, ^2Department of Biochemistry, ^3Department of Pharmacology, Center in Molecular Toxicology, and the Vanderbilt Cancer Center, Vanderbilt University School of Medicine, Nashville, TN 37232, USA
^4To whom correspondence should be addressed

Malondialdehyde (MDA*), a product of lipid peroxidation, causes mutations in bacterial and mammalian cells and cancer in rats. MDA reacts with deoxynucleosides in vitro and the monomeric adduct of MDA with deoxyguanosine (M,G-dR) is the major adduct. M,G-dR has been detected in rat and human liver. Random mutagenesis studies with MDA-modified DNA and recent 32P-postlabeling studies indicate that in addition to M,G-dR, adducts to deoxyadenosine may also be formed. We have utilized liquid chromatography coupled with electrospray ionization tandem mass spectrometry to characterize an N^6-oxopropenyl-2'-deoxyadenosine adduct (M,A-dR) in calf thymus DNA modified with MDA.

Malondialdehyde (MDA*) is produced by enzymatic and non-enzymatic breakdown of prostaglandin endoperoxides and is an end product of lipid peroxidation (1-3). MDA is mutagenic to bacteria and mammalian cells and is carcinogenic to rats (4-6). Thus, it is a genotoxic agent produced endogenously in humans which may contribute to the development of cancer. MDA forms a number of adducts with nucleosides in vitro and one of these adducts, pyrimido[1,2-α]purine-10(3/α)-one (M,G) (Figure 1), is the major adduct at physiological pH (7-9). This adduct has been identified in MDA-treated calf thymus DNA, rat liver DNA and human liver DNA. Recently, we quantitated M,G-2'-deoxyribose (M,G-dR) in rat livers (10) and disease-free human livers (11) using gas chromatography/electron capture negative chemical ionization mass spectrometry. Subsequently, liquid chromatography/electrospray ionization tandem mass spectrometry (LC/ESI MS/MS) was used to establish unequivocally that M,G-dR is a constituent of human liver (12). Although MDA also forms minor oligomeric adducts with adenosine (13) (Figure 1) and cytidine (14) in vitro, M,G-dR is the only adduct that has been detected in MDA-modified calf thymus DNA. Recently, MDA-DNA adducts, presumably arising from the reaction of MDA with 2'-deoxyadenosine, have been detected in vivo using 32P-postlabeling (15,16). However, the structures of these adducts were not established. In the present report, we describe the use of ESI MS/MS coupled with HPLC to characterize a malondialdehyde-2'-deoxyadenosine (M,A-dR) adduct in calf thymus DNA modified with MDA.

β-Benzoyloxyacrolein (BBA) was synthesized as described previously (17). Calf thymus DNA was purified by ribonuclease and proteinase K treatment followed by organic solvent extraction (18). The A260/A280 ratio was always >1.85, indicating minimal protein contamination. Ribonuclease T1, ribonuclease A, phosphodiesterase I, alkaline phosphatase, micrococcal nuclease and proteinase K were purchased from Sigma Chemical Co. (St Louis, MO). All other chemicals were of reagent grade, and all solvents were HPLC grade.

M,A-dR was synthesized according to the reported procedure (13) after minor modifications. Tetraedioxypropane (400 mg) was treated with 1 N HCl (0.5 ml) for 10 min at room temperature and neutralized to pH 4.0 with aqueous NaOH. 2'-Deoxyadenosine (50 mg) was added to the reaction mixture and stirred at room temperature for 24 h. The reaction mixture was extracted with dichloromethane (3 × 2 ml) and the product was purified on a reverse-phase ODS semipreparative (250 × 10 mm i.d.; 5 μm) column under isocratic conditions using a mobile phase of acetonitrile/water (15:85 v/v) at a flow rate of 2.5 ml/min. M,A-dR eluted at 17.5 min and corresponding depurinated product, M,A, eluted at 14 min as a minor peak.

1H NMR (300 MHz, D2O): δ 2.43 (m, 1H, H-2,2'), 2.69 (m, 1H, H-2,2'), 3.68 (m, 2H, H-5,5'), 4.03 (m, 1H, H-4'), 4.50 (m, 1H, H-3'), 5.81 (dd, 1H, J = 8.7, 13.5 Hz, H-b), 6.35 (t, 1H, J = 6.8 Hz, H-1'), 8.32 (s, 2H, H-2, H-8), 8.45 (d, 1H, J = 13.5 Hz, H-c), 9.12 (d, 1H, J = 8.7 Hz, H-a).

ESI MS: MH^+ m/z 306; BH2^+ m/z 190; ESI MS/MS: product ions of m/z 306, 190 and 162.

Freshly prepared BBA (25 mM final concentration) was added to a solution of calf thymus DNA (0.5 mg) in phosphate buffer (50 mM, pH 6.5, 1 ml). The reaction mixture was incubated at 37°C for 8 h. Modified DNA was recovered by ethanol precipitation and stored at −80°C.

For enzymatic hydrolysis, DNA was dissolved in 50 mM Tris buffer (1 ml, pH 8.9) containing 10 mM calcium chloride and 10 mM magnesium chloride. Micrococcal nuclease was added to a solution of calf thymus DNA (0.5 mg) in phosphate buffer (50 mM, pH 6.5, 1 ml). The reaction mixture was incubated at 37°C for 8 h. Modified DNA was recovered by ethanol precipitation and stored at −80°C.

For enzymatic hydrolysis, DNA was dissolved in 50 mM Tris buffer (1 ml, pH 8.9) containing 10 mM calcium chloride and 10 mM magnesium chloride. Micrococcal nuclease was added to a solution of calf thymus DNA (0.5 mg) in phosphate buffer (50 mM, pH 6.5, 1 ml). The reaction mixture was incubated at 37°C for 8 h. Modified DNA was recovered by ethanol precipitation and stored at −80°C.

© Oxford University Press
Reconstructed ion chromatograms of \( m/z \) 252 (dA), 268 (dG), 304 (M,G-dR) and 306 (M,A-dR) are shown in Figure 2. BBA showed the presence of a component eluting at 11.54 min. This and 304 showed the presence of dC, dT, dA, dG and M,G-dR. The reconstructed ion chromatograms for \( m/z \) 228, 243, 252, 268 and 304 correspond to MH\(^+\) ions of dA, dG, M,G-dR and M,A-dR respectively.

The hydrolysate from MDA-modified calf thymus DNA was analyzed by LC/ESI CNL MS/MS. The reconstructed ion chromatograms for \( m/z \) 252, 268, 304 and 306 were obtained from precursor ions of M,A-dR and M,G-dR (upper panel, \( m/z \) 306 → 190) and M,G-dR (middle panel, \( m/z \) 304 → 188) respectively. The total reconstructed ion chromatogram is shown in the lower panel.

The LC/ESI MS and MS/MS studies were performed on a Finnigan TSQ-7000 interfaced with an HP 1090 series II liquid chromatograph. Solvent A was 5 mM aqueous ammonium acetate containing 0.04% acetic acid and solvent B was 5 mM methanolic ammonium acetate containing 0.04% acetic acid. LC was performed on a narrow bore C8 column (Zorbax, Mac Mod Analytical Inc., Chadds Ford, PA; 100 × 2.1 mm i.d.; 5 \( \mu \)m) maintained at 40°C with a flow rate of 200 µl/min. A linear gradient was run as follows: 0 min, 0% B; 5 min, 0% B; 10 min, 70% B; 12 min, 95% B; 15 min, 95% B; 20 min, 0% B; 25 min, 0% B. Nitrogen gas (Liquid Air, Walnut Creek, CA) was used as sheath (70 p.s.i.) and auxiliary (20 p.s.i.) gas to assist with nebulization. An ESI spray voltage of 4.5 kV was applied to the spray needle. The capillary was heated to 200°C in order to provide optimal desolvation. The ESI interface and mass spectrometer parameters were optimized to obtain maximum sensitivity without sacrificing unit resolution. Argon (Liquid Air) was used as the collision gas. Constant neutral loss (CNL) scanning was performed by setting a neutral offset of 116 daltons (corresponding to the loss of the deoxyribose moiety) between the first and the third quadropole. The collision cell pressure and skimmer pump pressure were set at 8.47 × 10\(^{-5}\) Torr respectively. The CID offset of the octapole lens was used to cause ‘in-source’ dissociation of the analyte molecule.

The chromatogram of MDA-modified calf thymus DNA hydrolysate was analyzed by LC/ESI CNL MS/MS. The reconstructed ion chromatograms for \( m/z \) 228, 243, 252, 268 and 304 showed the presence of dC, dT, dA, dG and M,G-dR respectively (12). The presence of M,A-dR was detected by reconstructing ion chromatograms for \( m/z \) 306 (MH\(^+\)). This showed the presence of a component eluting at 11.54 min. Reconstructed ion chromatograms of \( m/z \) 252 (dA), 268 (dG), 304 (M,G-dR) and 306 (M,A-dR) are shown in Figure 2. BBA is more reactive than MDA and extent of DNA modification is greater, which facilitates study of minor adducts like M,A. Recently, we have also been able to detect this adduct from NaMDA-modified DNA.

To enhance the signal-to-noise ratio a selected reaction monitoring experiment was performed by monitoring MH\(^+\) → BH\(^+\) transitions of \( m/z \) 304 → 188 (for M,G-dR) and \( m/z \) 306 → 190 (for M,A-dR) (Figure 3). Although the comparison of intensities in the selected reaction monitoring is not quantitative, the experiment indicates that M,A-dR levels in MDA-modified calf thymus DNA were significantly lower than M,G-dR levels.

For further elucidation of the structure, product ion spectra were obtained from precursor ions of M,A-dR and M,G-dR (\( m/z \) 306 and 304 respectively). The LC MS/MS spectrum of \( m/z \) 306 at −22 eV collision offset showed a distinct peak for BH\(^+\) ion at \( m/z \) 190 (Figure 4a). Another ion was observed at \( m/z \) 162 representing the loss of 28 daltons (loss of C=O). The LC MS/MS spectrum of \( m/z \) 304 at −22 eV collision offset showed a distinct peak for BH\(^+\) ion at \( m/z \) 188.

One of the attractive features of electrospray ionization is the ability to carry out source-induced dissociation (SD) by increasing the octapole offset voltage. This provides a means to generate structurally informative MS/MS spectra. Studies with M,G-dR have shown that at high octapole offset voltages the BH\(^+\) ion at \( m/z \) 188 was the major ion in the mass spectrum (12). Source-induced dissociation in this region is thought to result from increased collisions of MH\(^+\) ions with neutral molecules as they travel between the skimmer and the octapole assembly. LC/ESI source-induced MS/MS analysis of M,G-dR and M,A-dR was performed by adjusting the octapole offset to give maximal production of the BH\(^+\) ions at \( m/z \) 188 and 190, while minimizing the loss in total ion current. CID and MS/MS analysis of \( m/z \) 188 and 190 was then performed with an octapole offset of −52 eV. It was evident from the ESI SD/MS/MS spectrum that significant C−C cleavages of the base adduct had occurred. Major product ions for \( m/z \) 190 (M,A) were observed at \( m/z \) 162, 135, 119 and 94. The ion at \( m/z \) 162 results from the loss of C=O and at \( m/z \) 135 results from the loss of the aliphatic side chain.
The present results indicate that M)|A-dR is the principal MDA-deoxyadenosine adduct in double-stranded DNA. Wang noted that deoxyadenosine may be important premutagenic lesions. G —» T transversions, A —» G transitions and C —» T transitions) 70% of the mutations were base-pair substitutions (mainly following replication in (28). Approximately important role in mutagenesis and carcinogenesis (19-27). There is a growing realization that adducts produced during exposure to endogenous carcinogens produced during normal metabolism, oxidative stress and chronic inflammation play an important role in mutagenesis and carcinogenesis (19-27). Random modification of single-stranded M13MB102 DNA by MDA induces an ~10-fold increase in mutation frequency following replication in Escherichia coli (28). Approximately 70% of the mutations were base-pair substitutions (mainly G → T transversions, A → G transitions and C → T transitions) and the remainder were frameshifts and large deletions. The occurrence of A → G transitions implies that MDA adducts to deoxyadenosine may be important premutagenic lesions. The present results indicate that M)|A-dR is the principal MDA-deoxyadenosine adduct in double-stranded DNA. Wang and Liehr recently reported the detection by 32P-postlabeling of two adducts in liver and kidney DNA from hamsters that they propose to arise from MDA modification of deoxyadenosine residues (15,16). The amounts of material available for post-labeling analysis preclude spectroscopic characterization of the adducts so it is uncertain if one of them is M)|A-dR or a decomposition product formed during the multiple enzymatic digestions or chromatographic elutions associated with post-labeling analysis.

Acknowledgements
This work was supported by the A.B.Hancock Jr Memorial Laboratory for Cancer Research, research grant CA47479 and center grant ES0267 from the National Institutes of Health.

References


Received on December 7, 1995; revised on January 26, 1996; accepted on January 30, 1996