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Detection and Characterization of DNA Adducts at the Femtomole Level by Desorption Ionization Mass Spectrometry

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Current methodologies for the detection and isolation of carcinogen–DNA adducts have advanced beyond the capabilities of the methods used to elucidate their structures. This difficulty seriously limits the potential use of DNA–carcinogen adducts in human dosimetry. We have investigated two general strategies for the analysis of model arylamine–nucleoside adducts using desorption ionization mass spectrometry (MS). Using fast atom bombardment MS–MS with constant neutral loss scans, we can identify the protonated molecule of derivatized adducts in samples as small as 1 pmole, and then apply daughter ion MS–MS scans to obtain structure-specific fragmentation. Using this strategy we have differentiated adducts having the same carcinogen and different bases [e.g., *N*-(deoxyadenosin-8-yl)-4-aminobiphenyl and *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl] or the same base and different carcinogens [e.g., *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl and *N*-(deoxyguanosin-8-yl)-2-aminofluorene]. In the second approach we used laser desorption time-of-flight MS to obtain spectra from adduct samples as small as 20 fmole. These data indicate that MS can be used for the analysis of very low (picomole–femtomole) levels of nucleoside adducts, including isomers, and that desorption ionization MS and MS–MS have significant potential for applications in human dosimetry.

Introduction

The analysis of carcinogen–DNA adducts has been proposed as a means of evaluating the extent of human exposure to specific carcinogens (*1*). Unfortunately, most analytical methods with sufficient sensitivity to detect adducts at the levels expected in humans require authentic samples for use as standards. The characterization of unknown adducts from human subjects will likely require the development of mass spectrometric methodologies with greater sensitivity than have thus far been demonstrated. Recently, Annan et al. (*2*) reported the detection of picomole levels of trimethylsilyl (TMS) derivatives of nucleoside–carcinogen adducts based on fast-atom bombardment (FAB) ionization and MS or MS–MS (daughter ion) analysis. We have investigated the use of FAB ionization with constant neutral loss (CNL) scans to screen samples for carcinogen–nucleoside adducts (*3*) as well as their TMS derivatives (*4*). Preliminary evidence suggests that FAB ionization used in conjunction with MS–MS (daughter ion scans) also provides structure-characteristic data regarding adducts, including nonidentical spectra for isomers (*2,4*).

In this paper we report two strategies for the analysis of model arylamine-nucleoside adducts at the femtomole to picomole level. Our first approach involves the use of FAB ionization and CNL scans of TMS derivatives for adduct identification followed by FAB–MS–MS for structure elucidation. Our alternative approach, laser desorption (LD)–time-of-flight (TOF) MS, shows greater sensitivity, but typically gives less information regarding adduct structures.

Experimental

Derivatization Procedure. Adducts were silylated by adding *N*-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) directly to the dry sample and heating at 60 °C for 1–2 hr. Mass spectral analyses were performed directly with the derivatization solutions.

FAB Mass Spectrometry. Samples (0.5–1.0 μL) were applied to a copper target via a syringe after application of 1–2 μL of thioglycerol FAB matrix liquid. Spectra were obtained using a Finnigan MAT TSQ-70 with an Ion Tech gun. FAB ionization was effected using xenon atoms accelerated to 8–10 KeV.

FAB–MS–MS. Constant neutral loss scans were acquired using the Finnigan MAT TSQ 70 as described above for FAB MS. To obtain a constant neutral loss scan, the first and last quadrupoles, Q1 and Q3, were scanned at the same rate with a constant mass offset corresponding to the mass of a *bis*-trimethylsilyl derivatized deoxyribose (260 daltons). Daughter ion scans were obtained by fixing the mass passed by Q1 to correspond to the

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BH_2^+ ion while the mass of Q3 was scanned to detect the masses of all fragments that formed upon decomposition of the mass-selected parent ion. Both types of scans were acquired using argon as the collision gas to induce decomposition of the parent ion. Collision energies of 50 eV were used and collision cell pressures were adjusted to maximize the yield of daughter ions.

TOF–MS. LD–TOF mass spectra were acquired with a Vestec model 2000, using a large excess of a sinapinic acid matrix, a wavelength of 355 nm, and a power density of 1×10^6 W/cm^2 irradiating an area of approximately 0.126 mm^2.

Results and Discussion

For nonderivatized arylamine–nucleoside adducts, we have obtained reproducible FAB mass spectra for samples as small as 100 ng (Table 1). Typically, the protonated molecule and BH_2^+ ion are just detectable above the near continuous background signal attributed to the FAB matrix liquid. Comparable detection limits (500 ng) have been reported elsewhere (*2*). When the adducts were TMS derivatized, we obtained mass spectra at levels as low as 1 ng. For example, the FAB mass spectrum obtained using N-(deoxyguanosin-8-yl)-2-aminofluorene (dG-C8-AF) typically showed a protonated molecule for the *bis*- and *tris*-TMS derivatives (m/z 591 and 663) as well as a monosilylated BH_2^+ ion (m/z 403) and an unsilylated BH_2^+ fragment ion (m/z 331). Other arylamine adducts, *N*-(deoxyadenosin-8-yl)-4-aminobiphenyl (dA-C8-ABP), *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl (dG-C8-ABP), and N- (deoxyguanosin-8-yl)-2-acetylaminofluorene (dG-C8-AAF) showed similar behavior.

We have also investigated the use of CNL scans on TMS-derivatized carcinogen–nucleoside adducts. In these experiments a CNL corresponding to the loss of the *bis*-TMS derivatized deoxyribose was selected. Incomplete derivatization is not a difficulty with this method because the deoxyribose moiety of DNA adducts is readily derivatized, and these TMS groups are stable enough to allow analysis by FAB–MS (*5*). FAB CNL scans of MSTFA derivatized adducts were obtained from samples as small as 0.5 ng (1 pmole). However, the simplification of the spectra, based on removal of extraneous (nonanalyte) signals, may have greater significance than the reduction in the amount of sample needed for analysis. Typical of these results was the spectrum obtained using 1 ng of dG-C8-AAF. Signals at m/z 591 and 633 corresponding to loss of a *bis*-TMS derivatized deoxyribose (260 daltons) from the protonated molecules of the *bis*-TMS and *tris*-TMS derivatives of dG-C8-AAF were readily observed. Irreproducible results have thus far been observed at the 100 pg level; however, detection of adducts may be achieved at the 100 pg level using FAB ionization in the selected reaction monitoring (SRM) mode. With Q1 and Q3 set to pass only the TMS derivatized protonated molecule and BH_2^+ fragment ion, respectively, significant signals were observed from 5–10 pg of adduct, but not from the thioglycerol matrix. Although SRM is inherently limited in application to targeted or selected masses, it is a suitable methodology for quantitative analysis using isotope dilution MS, an application that we are currently investigating.

Table 1. Detection limits for the analysis of carcinogen–nucleoside adducts using various desorption ionization methodologies.

Ionization method	Derivative	Scan type	Detection limit, ng[a]
FAB	None	MS	100
FAB	TMS	MS	1
FAB	TMS	CNL	0.5
FAB	TMS	SRM	0.01
LD–TOF	None	MS	0.01

Abbreviations: FAB, fast-atom bombardment; LD–TOF, laser desorption–time of flight; MS, full scan mass spectrum; CNL, constant neutral loss mass spectrum; SRM, selected reaction monitoring.

[a]Defined as the smallest amount of adduct with which we have obtained a signal-to-noise level of about 3 to 1 for the listed scan type.

We have also investigated the use of MS–MS with daughter ion scans as a probe of the structure of carcinogen-nucleoside adducts. Similar methods for specific carcinogen-DNA adducts have been reported using linked scanning MS techniques (*2*). The typical procedure for characterizing analyte structures via desorption ionization and MS–MS involves collision-induced dissociation (CID) of the analyte protonated molecule. CID produces an intense BH_2^+ for arylamine–nucleoside adducts and little additional evidence regarding the adduct's structure. TMS derivatization complicates interpretation because an adduct containing multiple TMS groups typically includes more than one isomeric form. For this reason, the specific daughter ions probed via FAB–MS–MS were underivatized BH_2^+ fragments rather than TMS-containing species, even when TMS derivatized adducts were used. Using this approach, problems associated with isomeric forms were avoided. Fortuitously, CID spectra from the BH_2^+ fragment ions also show considerably more structurally characteristic fragmentation than the CID spectra of the protonated molecules (*2*).

FAB–MS–MS daughter ion spectra were obtained from the BH_2^+ ion of dG-C8-AF and dG-C8-ABP derivatized using MSTFA. The same neutral losses (17, 42, 44, 70, 72, 111, 112, 124, 139, 151, and 166 daltons) were observed for both of these C-8-substituted dG adducts. This was attributed to fragmentation in which the carcinogen was retained within the part of the molecule with the positive charge. Subsequent experiments using deuterated adduct (dG-C8-d_9ABP) confirmed retention of the carcinogen moiety in these fragments. Structure specificity attributed to the C8-substituted deoxyguanosine moiety was also observed from fragment ions with the same masses (rather than resulting from identical neutral losses). Fragment ions at m/z 95, 112, 123, and 140, observed with both dG-C8-AF and dG-C8-ABP, were attributed to fragments from which the carcinogen moiety has been lost. With an adduct of a different nucleoside, having the same carcinogen and similar carcinogen-base bonding (dA-C8-ABP), most of the dG-characteristic losses associated with retention of the carcinogen moiety in the neutral fragment were not observed. However, some ions were common to both the ABP adducts (i.e., m/z 207, 195, 180, 168, 153). These are attributed to specificity for the carcinogen moiety resulting from loss of most of the base. The structure specificity of these low-energy CID spectra are very similar to results obtained using high-energy CID with a magnetic sector instrument (*2*).

The smallest amount of adduct for which we have obtained mass spectra was accomplished using LD with a TOF MS. The high sensitivity of LD–TOF MS, compared to the other MS methods we have studied, is attributed primarily to the use of an integrating MS (TOF) capable of detecting all the ions produced during each ionization/vaporization step. The spectrum for 3-(deoxyguanosin-N^2-yl)-2-acetylaminofluorene, obtained by

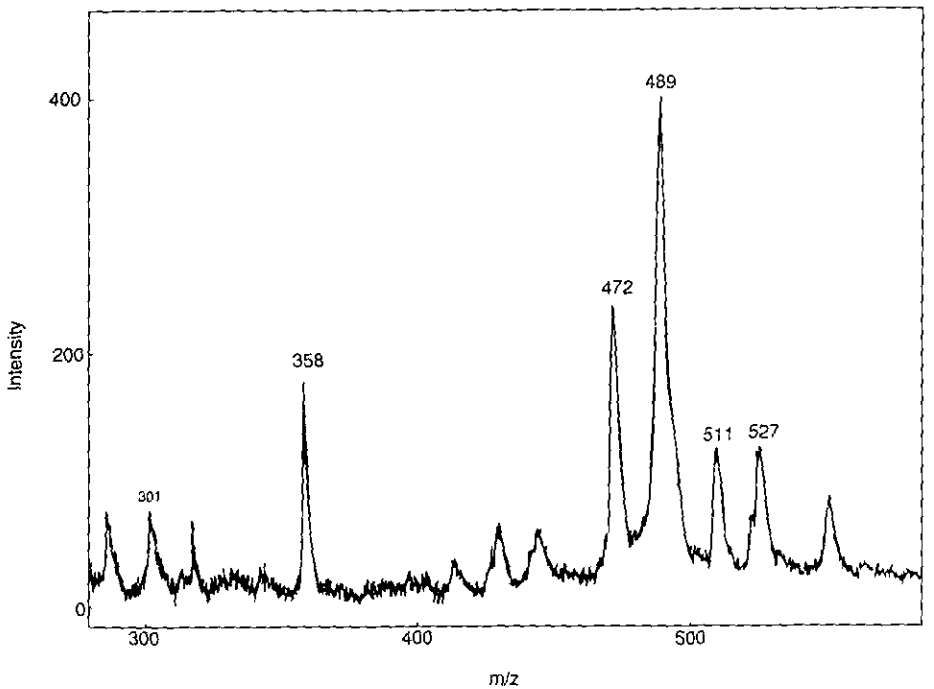

FIGURE 1. The laser desoption-time-of-flight mass spectrum obtained from 20 fmole of 3-(deoxyguanosin-N^2-yl)-2-acetylaminofluorene.

adding the signals obtained using 100 of 500 laser shots from a single probe loading of 20 fmole, is shown in Figure 1. Signals for the protonated molecule, $[M+Na]^+$, and $[M+K]^+$ were readily observed at m/z 489, 511, and 527 respectively. Although the LD-TOF methodology offers little promise for structure characterization at present, the LD ionization step coupled with fourier transform mass spectrometry (FT-MS) may allow more complete characterization of nucleoside-carcinogen adducts.

Conclusion

The minimum amount of nucleoside-carcinogen adduct necessary for detection of the protonated molecule and BH_2^+ fragment ion in full scans using TMS derivatization and FAB-MS was about 1 ng. In FAB-CNL and SRM experiments, the detection limits were 500 and 10 pg, respectively. CID spectra have the potential to allow structural features of adducts to be probed using FAB ionization. The lowest detection limit observed was obtained using LD with a TOF instrument. Although only a small portion of the target was irradiated, spectra were observed when samples as small as 10 pg were applied to the target. For adduct levels of 1 in 10^8 nucleosides, a detection limit of 10 pg (20 fmole) would make the analysis of tissue samples as small as 1 g feasible, assuming quantitative recovery in the preliminary isolation steps.

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