Low Energy Tandem Mass Spectrometry of Deoxynucleoside Adducts of Polycyclic Aromatic Hydrocarbon Dihydrodiol-Epoxides

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The use of fast-atom bombardment ionization-tandem mass spectrometry approaches, with collision energies on the order of 30–50 eV, was developed for the analysis of low picomole quantities of polycyclic aromatic hydrocarbon dihydrodiol-epoxide deoxynucleoside adducts. This strategy combines three experimental techniques: (1) product ion scans, (2) constant neutral loss scans, and (3) precursor ion scans. Product ion scans of the protonated molecule or the BH_2^+ ion that results from loss of the deoxyribose were dominated by fragments associated with cleavage of the sigma bond between the dihydrodiol-epoxide moiety and the nucleobase. Constant neutral loss scans were based upon the loss of deoxyribose (116 u) or the combined loss of the deoxynucleoside, water, and carbon monoxide (313 u); precursor ion scans utilized the latter fragment. The formation of trimethylsilyl derivatives increased the sensitivity of analysis, which allowed the simultaneous detection of DNA adducts in a mixture. (*J Am Soc Mass Spectrom* 1995, 6, 248–256)

Polycyclic aromatic hydrocarbons (PAHs) are a group of structurally related chemicals that constitute an important class of carcinogenic environmental pollutants [1]. These compounds, which are produced during combustion processes, are metabolized into electrophilic derivatives, typically dihydrodiol-epoxides, that are capable of reaction with DNA to elicit mutagenic and carcinogenic responses [2]. The analysis of carcinogen-DNA adducts that result from PAH dihydrodiol-epoxides or electrophilic derivatives from other classes of carcinogens has been proposed as a means of evaluation of the extent of human exposure to specific carcinogens [3]. DNA adduct analyses are complicated, however, by the low frequency of DNA adduction that occurs in vivo. In experimental animals, for example, only about 10–100 pmol of adduct per milligram of DNA are normally formed following treatment with tumorigenic doses of carcinogens. This problem is further complicated by the fact that DNA is a minor component (about 0.1% by weight) of tissue. In addition, humans usually are exposed to smaller amounts of carcinogens than those used with experi-

mental animals, with resultant lower levels of DNA adduction [4].

Several techniques, which include immunoassays [5], fluorescence assays [6], and ^{32}P -postlabeling [7], have been developed for the analysis of small quantities of carcinogen-DNA adducts. Although they are highly sensitive, these methods do not permit structure elucidation, which is a severe limitation when there is uncertainty with regard to the nature of the carcinogen exposure. Mass spectrometry has the potential to provide structural information and, as such, has played an important role in the structural elucidation of covalently modified nucleic acids [8].

High resolution electron impact mass spectrometry (EI-MS) has been used to determine the structures of several PAH dihydrodiol-epoxide DNA adducts [9]. However, the extreme polarity and thermal lability of these adducts necessitated the formation of methyl or silyl ethers prior to analysis via electron impact (EI) ionization. Because of the combined sensitivity losses associated with the use of high resolution and TMS derivatization, alternatives to the high resolution EI-MS method for measurement of adduct levels in human DNA warrant investigation, particularly for analyses that involve unknown carcinogen-DNA adducts.

Significantly less work has been reported on the tandem mass spectrometry of PAH dihydrodiol-epoxide DNA adducts. A deoxyguanosine adduct of

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anti-benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide (*anti*-BaPDE) was characterized by tandem mass spectrometry methods that used field desorption (FD) ionization and collision-induced dissociation (CID) under high collision energy (8 keV) conditions [10, 11]. The fragment ions generated in the presence of the helium collision gas were readily interpretable in terms of the assigned structure and were similar to fragment ions observed in EI mass spectra of the permethylated adduct [9]. Fast-atom bombardment (FAB) product-ion mass spectrometry studies of BaPDE adducts recently have been conducted via a VG Analytical (Fisons, Danvers, MA) ZAB-T four-sector (BEBE) mass spectrometer [12]. The product ion spectra for these adducts were dominated by ions derived from the carcinogen moiety. Other tandem mass spectrometry analyses of dihydrodiol-epoxide adducts have been limited primarily to studies that concern the site of carcinogen attachment to the nucleobase [13, 14]. A relatively limited number of tandem mass spectrometry studies have been conducted with derivatized PAH-DNA adducts; however, trimethylsilyl (TMS) derivatives of deoxynucleoside-carcinogen adducts showed surprising sensitivity by fast-atom bombardment-mass spectrometry (FAB-MS), to an extent that appeared to be greater than EI-MS [15, 16].

In this work, we report the development of FAB tandem mass spectrometry methods for the analysis of PAH dihydrodiol-epoxide deoxynucleoside adducts. This work, which is an extension of our previous studies with arylamine-DNA adducts [17], combines three experimental approaches: (1) constant neutral loss scans, (2) product ion scans of the protonated molecule or the BH_2^+ ion that results from loss of deoxyribose, and (3) precursor ion scans. We show that the sensitivity for analysis is increased by formation of TMS derivatives, which allows detection of PAH dihydrodiol-epoxide deoxynucleoside adducts at low picomole levels. In addition, we demonstrate that FAB tandem mass spectrometry, especially of derivatized adducts, provides a rapid, sensitive, and selective method for the analysis of complex mixtures, often with minimal, if any, sample cleanup.

Experimental

Materials

The following PAH dihydrodiol-epoxides were purchased from the NCI Chemical Carcinogen Reference Standard Repository (Midwest Research Institute, Kansas City, MO): *anti*-benzo[*k*]fluoranthene-*trans*-8,9-dihydrodiol-10,11-epoxide (*anti*-benzo[*k*]fluoranthene dihydrodiol-epoxide), *anti*-benzo[*b*]fluoranthene-*trans*-9,10-dihydrodiol-11,12-epoxide (*anti*-benzo[*b*]fluoranthene dihydrodiol-epoxide), *anti*-chrysene-*trans*-1,2-dihydrodiol-3,4-epoxide (*anti*-chrysene dihydrodiol-epoxide), *anti*-benz[*a*]anthracene-*trans*-3,4-dihydrodiol-1,2-epoxide (*anti*-benz[*a*]anthracene

dihydrodiol-epoxide), *anti*-dibenz[*a,c*]anthracene-*trans*-10,11-dihydrodiol-12,13-epoxide (*anti*-dibenz[*a,c*]anthracene dihydrodiol-epoxide), and *anti*-BaPDE. *N*-Trimethylsilylimidazole in pyridine (TMSI; Tri-Sil "Z") was acquired from Pierce Chemicals (Rockford, IL). 7,8,9,10-Tetrahydroxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene was prepared by treating *anti*-BaPDE with dilute hydrochloric acid for 18 h at 37 °C.

Adduct Synthesis and Derivatization

Covalent modification of DNA by dihydrodiol-epoxides of PAHs. To 1.2 mg of salmon testes DNA (Sigma Chemical Co., St. Louis, MO) in 1 mL of 5-mM Bis-Tris-HCl (Sigma), 0.1-mM EDTA, pH 7.1, was added 159 μg of *anti*-BaPDE in 107 μL of tetrahydrofuran. The mixture was allowed to react at 37 °C for 24 h and then was extracted with 1 mL of redistilled phenol saturated with an equal volume of 50-mM Bis-Tris-HCl, 1-mM EDTA, pH 7.1. Additional extractions were conducted with a mixture of 500 μL of phenol and 500 μL of Sevag (chloroform:isoamyl alcohol, 24:1), and then 1 mL of Sevag. To the aqueous phase was added 100 μL of 5-M NaCl and 1.1 mL of cold ethanol. The precipitated DNA was collected by centrifugation, washed twice with 70% ethanol, and added to 1 mL of 5-mM Bis-Tris-HCl, 0.1-mM EDTA, pH 7.1. The level of modification, as determined by UV spectrometry, with an $\epsilon_{347} = 29,000 \text{ mM}^{-1}$ [18], was 1.96%. The modified DNA was hydrolyzed as previously described [19] and the adducts were purified by high performance liquid chromatography (HPLC) with a $3.9 \times 300\text{-mm}$ $\mu\text{Bondapak C}_{18}$ column (Waters Associates, Milford, MA) and a 30-min linear gradient of 20–100% methanol at a flow of 2 mL/min. A 500-MHz ^1H -NMR spectrum of the major adduct was obtained and it was consistent with *N*²-deoxyguanosine substitution at C10 of *anti*-BaPDE. Similar procedures were used for reactions with the other PAH dihydrodiol-epoxides. The levels of modification from these dihydrodiol-epoxides were estimated by UV spectrometry by using the molar absorptivity value for the dihydrodiol-epoxides provided by the supplier.

Reaction between nucleosides with anti-BaPDE. To 10 mg of deoxyguanosine (U.S. Biochemical, Cleveland, OH; 35 μmol) in 500 μL of dimethylsulfoxide (DMSO) was added 60 μg of *anti*-BaPDE (0.2 μmol) and 10 μL of triethylamine. After 24 h at 37 °C, the reaction mixture was evaporated, 1 mL of water was added, and the mixture was extracted three times with diethyl ether to remove residual *anti*-BaPDE and degradation products. The aqueous phase was then applied to a C-18 Sep-Pak (Waters Associates), eluted with water to remove unreacted deoxyguanosine, and then eluted with methanol to recover the deoxyguanosine adduct in 18% yield. The adduct was further purified by HPLC, with a $\mu\text{Bondapak}$ column and the solvent

conditions described previously. The same procedure was used with deoxyadenosine and deoxycytidine. ^1H -NMR spectra were obtained for the deoxyadenosine and deoxycytidine adducts and both were consistent with exocyclic amine substitution at C10 of *anti*-BaPDE.

Reaction between nucleoside bases and *anti*-BaPDE. To 12.7 mg of guanine (Sigma; 84 μmol) in 1 mL of DMSO was added 200 μg of *anti*-BaPDE and 30 μL of triethylamine. Because the guanine was insoluble, the reaction was heated to 60 $^\circ\text{C}$. HPLC analysis after 24 h indicated the formation of a product in very low yield. Due to the low yield, the adduct was not isolated and FAB tandem mass spectrometry was performed directly on the reaction mixture. The same procedure was used with adenine and cytosine, except that the reactions were conducted at 37 $^\circ\text{C}$ for 24 h.

Derivatization procedures. Adducts were silylated by adding 10 μL of TMSI to 1 μg of dry sample and heating at 65 $^\circ\text{C}$ for 2 h. The sample was then dried *in vacuo* and diluted with DMSO prior to analysis. When modified DNA was analyzed, 100 μL of TMSI was added to the hydrolyzed samples.

Mass Spectrometry

After application of 1–2 μL of thioglycerol FAB matrix liquid, 0.5–1.0 μL of a solution that contained the sample was applied directly to a copper target with a syringe. Spectra were obtained via a Finnigan-MAT (San Jose, CA) TSQ-70 with an Ion Tech (Middlesex, UK) gun. FAB ionization was effected by using xenon atoms accelerated to 8–10 keV. Scans were obtained from approximately 50–700 u under control of the TSQ-70 data system. Constant neutral loss scans were acquired by scanning the first and last quadrupoles (Q1 and Q3) at the same rate, with a constant mass offset that corresponded to either the mass of the deoxyribose moiety (116 u) or the loss of 313 u. Product ion scans were obtained by fixing the mass passed by Q1 to correspond to the protonated molecule or the BH_2^+ ion, whereas Q3 was scanned to identify the masses of all the fragments formed by decomposition of the selected precursor ion. Precursor ion scans were obtained by selecting, with Q3, a specific product ion, whereas the first analyzer (Q1) was scanned to select all precursor ions of that fragment. All scans were acquired by using argon as the collision gas. Collision energies of 30–50 eV were used and collision cell pressures were varied to maximize the yield of useful product ions.

Results and Discussion

We previously demonstrated that chemical derivatization coupled with FAB-MS is a sensitive method for the analysis of arylamine–DNA adducts [17]. In a

continuation of this work, we now have determined that FAB-MS is a sensitive technique for the detection of DNA adducts derived from PAH dihydrodiol-epoxides. We also have established the types of structural information that can be obtained from FAB tandem mass spectrometry on these compounds.

Fast-Atom Bombardment Mass Spectrometry

Compared to the arylamine–deoxynucleoside adducts we studied previously [17], the PAH–deoxynucleoside adducts gave very poor FAB full scan mass spectra. Figure 1a shows a FAB mass spectrum from 100 ng of the deoxyguanosine adduct obtained from *anti*-chrysene dihydrodiol-epoxide. The ion of m/z 546 corresponds to the protonated molecule and that at m/z 430 to the BH_2^+ ion that results from loss of the deoxyribose moiety. Better results were obtained when the adducts were derivatized with TMSI; for example, the high mass region of a FAB mass spectrum for 5 ng of the *anti*-chrysene dihydrodiol-epoxide deoxyguanosine adduct after TMSI derivatization is shown in Figure 1b. The ions at m/z 646 and m/z 762 correspond, respectively, to *tris*-TMS derivatives of the BH_2^+ ion and the protonated molecule. The observation of a *tris*-TMS-modified BH_2^+ ion (m/z 646) 116 u lower in mass than the *tris*-TMS-derivatized protonated molecule indicates that for at least some of the *tris*-TMS-modified protonated molecules, all of the

Figure 1. FAB mass spectra of *anti*-chrysene dihydrodiol-epoxide deoxyguanosine adduct (a) before and (b) after derivatization with TMSI. Spectrum (a) was obtained with 100 ng of adduct, whereas spectrum (b) was recorded with 5 ng of TMSI-derivatized adduct.

TMS groups reside on the carcinogen and/or base. A more completely derivatized *pentakis*-TMS product would result from attachment of two, rather than no TMS groups to the deoxyribose moiety. *Bis* modification of the deoxyribose has been reported for arylamine-deoxynucleoside adducts [17], and was sometimes observed in this study based on the observation of weak protonated molecules for *pentakis*-TMS-derivatized products. For example, the deoxycytidine-BaPDE adduct gave an ion (spectrum not shown) that corresponds to a *pentakis*-TMS protonated molecule.

These results demonstrate the incomplete and sometimes variable TMS derivatization observed under the conditions used in this study. Attempts to use alternative derivatization strategies, including those already developed for arylamine-deoxynucleoside adducts, often resulted in decomposition of the PAH dihydrodiol-epoxide deoxynucleoside adducts. Nevertheless, except as noted below for the deoxyadenosine adduct of *anti*-BaPDE, an abundant *tris*-TMS-derivatized protonated molecule was observed for all of the TMS-modified PAH dihydrodiol-epoxide adducts. With the deoxyadenosine adduct of *anti*-BaPDE, neither a *tris*- nor a *pentakis*-TMS-protonated molecule was detected; only an ion that corresponds to the *tris*-TMS derivative of BH_2^+ was observed.

Product Ions

Underivatized adducts. To investigate the structural specificity of product ions from the adducts, and in particular, to determine if ions indicative of the site of base substitution could be identified, product ions were obtained from the protonated molecules of all the deoxyguanosine adducts. A FAB product ion spectrum of the protonated molecule (m/z 546) for the *anti*-benz[*a*]anthracene dihydrodiol-epoxide deoxyguanosine adduct is shown in Figure 2. These results are in good agreement with data reported previously for FD

Figure 2. FAB product ion spectrum of the protonated molecule (m/z 546) from 25 ng of *anti*-benz[*a*]anthracene dihydrodiol-epoxide deoxyguanosine adduct.

Scheme I. Proposed fragmentation pathway for the protonated molecules of PAH dihydrodiol-epoxide deoxynucleoside adducts. Initial cleavage of the C—N bond between the base and carcinogen gives a carcinogen carbocation (fragment 1). Subsequent loss of 18 and 28 u gives fragments 2 and 3, presumably due to loss of water and carbon monoxide, respectively. The latter fragment was typically the base peak. The "boat-like" conformation of the cyclohexane ring is shown below fragment 1.

and CID mass spectrometry of BaPDE deoxynucleoside adducts [10, 11]. Except for the greater abundance of BH_2^+ ion, the spectrum is typical of the results observed with the other PAH dihydrodiol-epoxide deoxyguanosine adducts. Fragment ions that correspond to loss of 267, 285, and 313 u from the protonated molecule were common to all of the spectra, and the proposed structures for these fragments, labeled 1, 2, and 3, respectively, are shown in Scheme I. An ion that corresponds to the protonated, unmodified base (guanine, m/z 152) also was observed from each of the deoxyguanosine adducts. Similar results, which include fragments that correspond to 1, 2, and 3, were observed with deoxyguanosine-BaPDE, deoxyadenosine-BaPDE, and deoxycytidine-BaPDE adducts; however, the mass loss that gives rise to ion 1 is dependent on the mass of the leaving group (nucleobase). With these deoxynucleoside adducts, ions that correspond to the protonated bases were noted at m/z 152 (guanine), m/z 136 (adenine), and m/z 112 (cytosine).

Many of the product ions from the protonated molecule are attributed to initial cleavage of the sigma bond between the carbon atom of the PAH dihydrodiol-epoxide and the exocyclic amine (N^2 for guanine) from the nucleobase. Formation of these ions can be explained based on the stability of carbocation structures (1, 2, and 3), initial cleavage of single rather than

multiple bonds, and differences in the bond strengths for C—N sigma bonds in aromatic amines as compared to aliphatic amines. This is accomplished most easily by using, as a frame of reference, the protonated molecule where the proton resides on the exocyclic nucleobase nitrogen. Migration of the pair of electrons that form the sigma bond between this nitrogen atom and the PAH carbon gives a stable carbocation that results from heterolytic cleavage of the C—N bond. The absence of a significant signal for the ion that results from the alternative heterolytic C—N cleavage between the nucleobase and the same exocyclic amine is consistent with the observation that for exocyclic amines, the C—N bonds between an aromatic carbon (pyrimidine or purine) and an exocyclic amine are typically stronger than the same bond between a cyclic aliphatic carbon and an exocyclic amine (bond energies ~4.4 and 3.7 eV, respectively [20]). After the initial formation of fragment 1, losses of water and then carbon monoxide result in the formation of two additional carbocations, fragments 2 and 3, respectively.

The protonated base ion results from cleavage of the same C—N bond that led to formation of ion 1 except that in this instance another hydrogen is transferred from the PAH to the amine nitrogen prior to cleavage. As a result the charge resides on the nucleobase side of the molecule. A proton transfer may be facilitated for the compounds investigated because of their conformation. The OH group on the opposite side of the cyclohexane ring is *cis* to the NH group and a "boat-like" conformation of the cyclohexane ring brings the NH and OH substituents on opposite sides of the cyclohexane ring into relatively close proximity (Scheme I). Such proximity and the resulting sharing of a proton was used to explain the relative stability (less fragmentation) of the *cis* isomer with respect to water loss in a study of protonated *cis*- and *trans*-1,4-cyclohexanediols [21]. In the current study the greater propensity for fragmentation, as opposed to less fragmentation from the *cis*-diols in ref 21, is attributed to the unequal sharing of a proton between nitrogen and oxygen. The proton affinity differences (typically about 1 eV) favor protonation of the nitrogen rather than the oxygen atom. Transfer of the charge-bearing proton (Scheme I) from oxygen to nitrogen would lead directly to fission of the PAH–nucleobase bond to give ion 1 (or the protonated base if the "extra" or charge-bearing proton happened to reside elsewhere on the nucleoside base).

The absence of an analogous ion that contains the carcinogen moiety and the protonated amine can be attributed in part to the relative bond strengths cited previously, but additional factors such as the energy–entropy requirements for proton transfer from the nucleobase to the exocyclic amine likely play a role as well. The formation of ions 1, 2, and 3 could occur directly from the MH^+ ion or indirectly via the BH_2^+ ion. The protonated base ion (m/z 152 in Figure 2) does not contain a deoxyribose sugar and is thus

attributed to fragmentation from an intermediate BH_2^+ fragment.

Model compounds for the BH_2^+ ions were synthesized by reaction of *anti*-BaPDE with guanine, adenine, and cytosine (Figure 3). A representative product ion spectrum for the guanine–BaPDE adduct is shown in Figure 4. The major fragments were the same as those observed from the protonated deoxynucleoside adducts, the structures of which were assigned in Scheme I.

RamaKrishna et al. [12] have conducted FAB product ion studies of BaPDE adducts of adenine and guanine with a four-sector mass spectrometer. The adducts, which were synthesized by electrochemical oxidation, were endocyclic *N*7-substituted products as compared to the exocyclic *N*²- (guanine) and *N*⁶- (adenine) substituted adducts shown in Figure 3. Although their spectra were acquired by using high energy conditions, the CID spectra were dominated by ions derived from the BaPDE moiety, with fragments 1, 2, and 3 (Scheme I) being readily apparent. These results suggest that product ion scans obtained from either low or high energy collisions provide structural information indicative of the molecular weights of the carcinogen and nucleobase, and perhaps some structural features. Unfortunately neither method provides fragment ions that are conclusive with regard to the site of carcinogen substitution.

Derivatized adducts. Figure 5a shows a product ion scan (m/z 570) of 25 ng of the *anti*-BaPDE deoxyguanosine adduct, whereas Figure 5b presents the product ion spectrum (m/z 786) of 5 ng of the same

Figure 3. Structures of *anti*-BaPDE guanine adduct (BaPDE-Gua), *anti*-BaPDE adenine adduct (BaPDE-Ado), and *anti*-BaPDE cytosine adduct (BaPDE-Cyt).

Figure 4. FAB product ion spectrum of the protonated molecule (m/z 454) from *anti*-BaPDE guanine adduct.

adduct after derivatization with TMSI. These data are typical of the results observed with all of the TMS-derivatized deoxynucleoside adducts. Although greater sensitivity was obtained following derivatization, the product ion spectrum of the derivatized sample showed fewer fragments. For example, fragments that correspond to ions 1 and 3 in Scheme I (or TMS-derivatized analogs) were not observed with the derivatized adduct. A fragment that corresponds to *bis*-TMS-derivatized fragment 2 was present (m/z 429 in Figure 5b); however, the most abundant fragment

ion is m/z 191. A product ion scan (not shown) of this m/z 191 ion gave a base peak at m/z 73, which was presumed to be $(\text{Si}(\text{CH}_3)_3)^+$. This suggests that the ion of m/z 191 corresponds to $[(\text{CH}_3)_3\text{SiO})_2\text{CH}]^+$. An ion at m/z 191 has been reported in the mass spectra of TMS derivatives of many polyhydroxy steroids; for example, a report by Brooks et al. [22] details a number of examples that show this and other rearrangements that involve the migration of TMS groups in mass spectra from trimethylsilyl ethers of steroids. Moreover, the structure of the m/z 191 ion was reported by Gustafsson et al. to be $[\text{TMSO}=\text{CH}-\text{OTMS}]^+$ [23].

With derivatized adducts, the m/z 191 fragment ion was also the base peak in product ion scans of the *anti*-BaPDE deoxyadenosine and deoxycytidine adducts. For the other dihydrodiol-epoxide deoxyguanosine adducts, the m/z 191 ion was prominent but the base peak was the protonated guanine base (m/z 152).

Constant Neutral Loss Scans

Underivatized adducts. A characteristic loss to produce a BH_2^+ ion has been observed in mass spectra obtained from protonated nucleosides and nucleotides [24–26], carcinogen-modified nucleosides [17, 27, 28], and silylated carcinogen-modified nucleosides [15, 16]. By extending this observation, we demonstrated the utility of constant neutral loss scans (116 u) for the determination of nucleoside adducts of arylamines [17]. Constant neutral loss scans are particularly useful for analysis of unknown adducts because they can be acquired prior to product ion spectra to determine the molecular weight of the adduct.

Although many of the PAH dihydrodiol-epoxide adducts used in this study showed BH_2^+ ions as unimolecular (Figure 1a) or collision-induced (Figure 2) fragments, others did not show loss of the deoxyribose, even with collisional activation of the protonated molecule (Figure 5a). In all cases, the intensity of this ion was much less than was expected based on results previously reported for arylamine- and arylamide-modified deoxynucleosides [16, 17]. Nevertheless, constant neutral loss scans that correspond to loss of 116 u were measured for all of the PAH dihydrodiol-epoxide adducts. For most of these adducts, loss of the deoxyribose from the protonated molecule, as well as the monosodium- and disodium-containing molecule ions, was observed (Figure 6a, for example). Another, more consistent type of constant neutral loss, based on the loss of 313 u, was observed for all of the deoxyguanosine-PAH dihydrodiol-epoxide adducts studied. This loss, which is attributed to consecutive losses of the components of water, carbon monoxide, and deoxyguanosine, gives rise to ion 3 in Scheme I. (The order of losses suggested in the scheme represents only one of several alternative mechanisms leading to an overall loss of 313 u.) Ion 3 is typically the base

Figure 5. FAB product ion spectra from the protonated molecule of *anti*-BaPDE deoxyguanosine adduct (a) before (m/z 570) and (b) after (m/z 786) derivatization with TMSI. Spectrum (a) was obtained with 25 ng of adduct, whereas spectrum (b) was recorded with 5 ng of TMS-derivatized adduct.

Figure 6. FAB constant neutral loss spectra of *anti*-benzo[*k*]fluoranthene dihydrodiol-epoxide deoxyguanosine adduct. Spectrum (a) follows the loss of 116 u; spectrum (b) follows the loss of 313 u. Similar results were obtained with the *anti*-benzo[*b*]fluoranthene dihydrodiol-epoxide deoxyguanosine adduct.

peak in product ion spectra of deoxyguanosine dihydrodiol-epoxide adducts. Consequently, when constant neutral loss scans were compared from identical samples, the loss of 313 u (Figure 6b) provided a more abundant signal than that observed with loss of 116 u (Figure 6a).

Derivatized adducts. Unsatisfactory results were obtained with the constant neutral loss spectra of TMS-derivatized PAH dihydrodiol-epoxide adducts. Although some adducts showed a loss of 116 u in product ion spectra (e.g., Figure 5b), this loss was not observed universally in product ion or constant neutral loss spectra. This can be attributed to alternative fragmentation pathways to form ions that correspond to 2 (Scheme I) or m/z 191. These results contrast with those previously obtained for TMS-derivatized arylamine adducts [17], where loss of 260 u (*bis*-TMS derivatized deoxyribose) was observed for all of the arylamine adducts studied. In the present study, the hydroxyl groups unique to the dihydrodiol-epoxides were derivatized more readily (or the attached TMS groups were retained more readily) than those on the deoxyribose. Thus, constant neutral losses of 260 u can be measured only from protonated molecules of *pentakis*-TMS derivatives, and these were not always observed.

Precursor Ion Scans

Underivatized adducts. Product ion spectra for *anti*-BaPDE adducts of deoxyguanosine, deoxyadenosine, and deoxycytidine showed a common base peak ion of m/z 257 (fragment 3 in Scheme I). These results suggested that if the identity of the PAH was known, precursor ion scans could be used to elucidate the identity of the deoxynucleoside that had been modified. Accordingly, when precursor ion spectra (257 u) were recorded for the three BaPDE adducts, protonated molecules were observed at m/z 570 (deoxyguanosine), 554 (deoxyadenosine), and 530 (deoxycytidine). A representative FAB precursor ion spectrum (257 u) for the *anti*-BaPDE deoxyadenosine adduct is shown in Figure 7. The detection limit for this approach was typically around 10 ng.

Derivatized adducts. With underivatized adducts, precursor ion scans were based upon the PAH moiety (i.e., fragment 3 in Scheme I). TMS-derivatized adducts proved to be of greater utility for precursor ion scans because the m/z 191 ion was not dependent on either the PAH or the deoxynucleoside. (This ion, m/z 191, has not been observed from TMS derivatives of other types of DNA adducts or from the TMS derivatization of biological and matrix blanks.) Thus, by using TMS-derivatized adducts, information could be obtained on both the PAH dihydrodiol-epoxide and the deoxynucleoside. As an example, Figure 8 shows a precursor ion scan from DNA that had been reacted with *anti*-BaPDE, enzymatically digested to deoxynucleosides, and then derivatized with TMSI. Two adducts were apparent: one was derived from deoxyguanosine, as evident from ions of m/z 858, 786, and 670, which represent the *tetrakis*- and *tris*-TMS derivatives of the protonated molecule and the *tris*-TMS derivative of BH_2^+ , respectively. The other adduct resulted from deoxyadenosine, as indicated by the ion of m/z 677, which results from the *tris*-TMS $BHNa^+$ derivative.

To determine the utility of this technique for the analysis of mixtures that contain deoxynucleoside adducts from different PAH dihydrodiol-epoxides,

Figure 7. FAB precursor ion spectrum (m/z 257) for *anti*-BaPDE deoxyadenosine adduct.

Figure 8. FAB precursor ion spectrum (m/z 191) of DNA reacted with *anti*-BaPDE. Following enzymatic hydrolysis to deoxynucleosides, the hydrolysate was derivatized with TMSI.

DNA was reacted with *anti*-BaPDE, *anti*-chrysene dihydrodiol-epoxide, and *anti*-dibenz[*a,c*]anthracene dihydrodiol-epoxide. The modified DNA was then hydrolyzed enzymatically to deoxynucleosides that were derivatized with TMSI. HPLC-UV analysis of the deoxynucleoside mixture before derivatization indicated the presence of the three deoxyguanosine adducts in a 4.2:5.8:1.0 ratio; however, only those adducts that resulted from reactions with *anti*-BaPDE and *anti*-chrysene dihydrodiol-epoxide were detected in the precursor ion scans (Figure 9). The ions of m/z 670, 786, and 858 corresponded, respectively, to the *tris*-TMS derivative of BH_2^+ and the *tris*- and *tetrakis*-TMS derivatives of the protonated molecule of the *anti*-BaPDE deoxyguanosine adduct. Likewise, the ion of m/z 646 and perhaps the weaker signals for m/z 762 and 834 may be attributed to the *tris*-TMS derivative of BH_2^+ and the *tris*- and *tetrakis*-TMS derivatives of the protonated molecule of the *anti*-chrysene dihydrodiol-epoxide deoxyguanosine adduct. In addition, an *anti*-BaPDE deoxyadenosine adduct was suggested by the

Figure 9. FAB precursor ion spectrum (m/z 191) of DNA reacted with *anti*-BaPDE, *anti*-chrysene dihydrodiol-epoxide (ChryDE), and *anti*-dibenz[*a,c*]anthracene dihydrodiol-epoxide (DBADE). Following enzymatic hydrolysis to deoxynucleosides, the hydrolysate was derivatized with TMSI.

Figure 10. FAB mass spectrum of DNA reacted with *anti*-BaPDE, *anti*-chrysene dihydrodiol-epoxide, and *anti*-dibenz[*a,c*]anthracene dihydrodiol-epoxide (DBADE). Following enzymatic hydrolysis to deoxynucleosides, the hydrolysate was derivatized with TMSI.

presence of a weak ion of m/z 677, which corresponded to the *tris*-TMS $BHNa^+$ derivative.

Constant neutral loss scans were obtained on the same mixture and only adducts obtained from *anti*-BaPDE and *anti*-chrysene dihydrodiol-epoxide could be identified (spectra not shown); however, a FAB full scan spectrum (Figure 10) showed a small fragment ion of m/z 696 that corresponded to the *tris*-TMS derivative of the BH_2^+ of the deoxyguanosine dibenz[*a,c*]anthracene dihydrodiol-epoxide adduct. The failure to detect this adduct in precursor ion or constant neutral loss scans is probably due to its low concentration relative to the other two deoxyguanosine adducts in the DNA sample.

Conclusions

These studies used three different experimental techniques (product ion, constant neutral loss, and precursor ion scans) to investigate PAH dihydrodiol-epoxide deoxynucleoside adducts. The results indicate that FAB tandem mass spectrometry has significant potential for the detection and structural characterization of PAH-dihydrodiol-epoxide as well as arylamine-DNA adducts, and the most appropriate technique depends on the specific adduct (e.g., class of carcinogen) and the information desired from the experiment. With a DNA adduct that results from a dihydrodiol-epoxide of an unknown PAH, for example, constant neutral loss scans based upon loss of 313 u (for deoxyguanosine adducts) proved to be a very sensitive method for PAH identification. Although this method may be applicable only to PAH dihydrodiol-epoxide adducts of deoxyguanosine, this particular deoxynucleoside is typically the major site for adduct formation in DNA [2].

In instances where the identity of the PAH dihydrodiol-epoxide is known, a precursor ion scan based upon a PAH fragment (i.e., fragment 3 in Scheme 1) can be used to identify the molecular weight of the

adduct and, hence, the deoxynucleoside. Alternatively, the mass of PAH dihydrodiol-epoxide initially can be determined by using constant neutral loss scans (313 or 116 u) and this can be followed by precursor ion scans to identify the deoxynucleoside. For adducts that result from carcinogens other than PAH dihydrodiol-epoxides, constant neutral loss scans of 116 u, which represents the loss of deoxyribose, can be used to determine the molecular weight of the adduct, and then product ion scans can readily distinguish arylamine from PAH dihydrodiol-epoxide adducts due to abundant losses (e.g., 313 u for the deoxy-guanosine-PAH adduct) from the dihydrodiol-epoxide adducts.

Because of the more extensive fragmentation observed from the protonated molecules of PAH dihydrodiol-epoxide adducts, it is not necessary to conduct product ion studies using BH_2^+ ions, as was the case with the arylamine adducts previously studied [17]. Preparation of TMS derivatives increased the sensitivity of adduct detection. Precursor ion scans of these TMS derivatives, by using a 191-u fragment, allowed simultaneous detection of several DNA adducts in a mixture. These results suggest potential applications of tandem mass spectrometry techniques to the identification of PAH dihydrodiol-epoxide derived carcinogen-DNA adducts.

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