Differentiation of Isomeric C8- and N²-Deoxyguanosine Adducts of 2-Acetylaminofluorene by Fast-Atom Bombardment and Tandem Mass Spectrometry

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Product-ion studies of source-produced ions corresponding to acetylated and nonacetylated N²- and C8-substituted aminofluorene adducts of deoxyguanosine were conducted to identify specific fragmentation pathways differentiating the isomers and to determine the influence of the acetyl group on the fragmentation of the arylamide modified deoxyguanosine adducts. The collision-induced dissociation spectra of the BH₂⁺ ion and other significant source-produced ions showed no evidence to suggest that ketene loss (deacetylation) resulted in significant alteration of the structure of the adducts. However, other significant ion formation processes, particularly loss of water from the N²-substituted arylamide did appear to require rearrangement, likely involving bond formation between the carcinogen moiety (acetyl group) and the N1 or N² position of the guanine base. The combined loss of ketene and water constitute a fragmentation pattern specific for the N²-arylamide, 3-(deoxyguanosin-N²-yl)-2-acetylaminofluorene. (*J Am Soc Mass Spectrom* 1994, 5, 58–63)

The analysis of carcinogen-modified nucleosides by mass spectrometry is motivated, in part, by the belief that DNA adducts play a critical role in the initiation of cancer. Indeed, the levels of adducts in DNA are regarded by many as indicators of the extent of exposure to genotoxins [1]. Because these adducts typically occur in humans at very low levels (as low as one base modification per 10¹⁰ nucleotides), they can be detected (and quantified) much more easily than identified [2–4]. Although negative-ion chemical ionization mass spectrometry studies of electrophore-derivatized alkylated bases have been accomplished at very low levels [5], the analysis of DNA modified with more complex carcinogens (i.e., arylamines) is usually accomplished by quantification of only the carcinogen moiety after isolation from the DNA [6]. While such an analytical methodology is sufficient for estimating the exposure to a given carcinogen(s), information regarding the position of carcinogen attachment on the DNA is lost. A methodology allowing individual nucleoside or nucleotide adducts to be analyzed is needed, both to validate quantitative measurements of total DNA adduct levels and to provide insight into mechanisms of carcinogenesis (metabolism and DNA adduct formation) implied by specific adduct structures.

Product-ion studies of carcinogen-modified nucleobases have been influenced by the potential for developing a tandem mass spectrometry (MS/MS) strategy for identification and perhaps quantification of trace levels of these compounds. Such studies could lead to methods for elucidating the structures of unknown DNA adducts at levels well below current detection limits. The potential benefits of such a methodology as well as the need to incorporate the specificity of mass spectrometry into methods for the determination of DNA adducts have been noted elsewhere [7]. To date, product-ion scans of modified nucleobases have been successfully used to provide confirmatory evidence regarding the identity of the nucleobase, as well as the bonding of the carcinogen-base [8]. However, elucidation of the structure of an unknown nucleoside or nucleotide adduct with a complex carcinogen, isolated from tissue DNA, has not been demonstrated by MS/MS. This application will require two significant improvements in MS/MS methodologies, namely increased sensitivity and a detailed knowledge of adduct- or base-specific product-ion fragmentation pathways. A more complete understanding of the processes associated with formation of product ions can be obtained by using instrumentation that is currently available, and will be addressed in this work by using arylamide adducts as model compounds.

The product-ion spectra of C8 substituted guanine-arylamine adducts have been studied in some detail [9–11]. For the limited number of arylamine adducts

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studied, the product ions from the BH_2^+ precursor appear to include a significant number of base-specific fragments [11]. However, only one N^2 -substituted arylamine {1-(deoxyguanosin- N^2 -yl)-2-aminophenanthrene, dGuo- N^2 -APhen} has been extensively characterized [9]. [Typically, arylamines/arylamides attach to the guanine (Gua) base in DNA either at the C8 or N^2 position.] For arylamide adducts, product-ion data in the literature are similarly limited to those for C8 substituted adducts [9, 11]. Herein we report low energy collision-induced dissociation (CID) (50 eV) studies that demonstrate the existence of product-ion pathways for an N^2 -substituted arylamide that are quite different than those from the C8 isomer and that may be specific for N^2 -substitution. Because the C8-substituted arylamide used in this study (*N*-(deoxyguanosin-8-yl)-2-acetylaminofluorene, dGuo-C8-AAF) was previously examined by using high energy CID and B/E scans [9] and by using low collision energies [11], direct comparison with previous experiments can be made. We also note evidence for decompositions from the N^2 -isomer involving interactions between the arylamide group and the guanine base.

Experimental

All fast-atom bombardment (FAB) product-ion spectra were acquired by using a Finnigan MAT TSQ-70 (San Jose, CA) equipped with an Ion Tech atom gun (Middlesex, England). FAB ionization was induced by Xe atoms accelerated to energies of 8–10 keV. Product-ion scans of the protonated molecule, the BH_2^+ , and other fragment ions were acquired by setting Q1 to the mass of the precursor ion while Q3 was scanned from the mass value set for Q1 down to m/z 50. The collision energy was set to 50 eV, and the pressure in the collision cell Q2 was approximately 0.7 to 0.8 mtorr.

The isomeric AAF-dGuo adducts were prepared for product-ion scans by derivatizing the deoxyribose hydroxyl groups with trimethylsilyl groups. This procedure was used because it is currently the most sensitive approach to analysis of deoxynucleoside adducts with static FAB ionization [9, 11]. An aliquot of solution containing 100 ng of the adduct was introduced into a 100- μ l conical vial and centrifuged under vacuum until dry. Two microliters of *N*-methyl-*N*-trimethyltrifluoroacetamide (MSTFA-Pierce, Co.) were then added to the residue and the mixture was heated for 1 hour at 55 °C. Normally all the solution recovered from the vial was analyzed in one or two FAB probe loadings.

The model adducts employed in this study, *N*-(deoxyguanosin-8-yl)-2-acetylaminofluorene (dGuo-C8-AAF) and 3-(deoxyguanosin- N^2 -yl)-2-acetylaminofluorene (dGuo- N^2 -AAF, Figure 1) were synthesized as described in refs 12 and 13, respectively. Both compounds were provided by Dr. Frederick A. Beland of the National Center for Toxicological Research.

***N*-(deoxyguanosin-8-yl)-2-acetylaminofluorene**

3-(deoxyguanosin- N^2 -yl)-2-acetylaminofluorene

Figure 1. The basis for numbering the atoms and position of substitution of compounds used in this study.

Results and Discussion

The strategy employed throughout this investigation was to activate the protonated carcinogen-modified base (the BH_2^+ ion) and other important source-produced fragment ions to provide evidence regarding isomer-specific fragmentation pathways, product-ion structures, and likely fragmentation mechanisms. The product-ion spectra of the BH_2^+ ion derived from dGuo-C8-AAF is dominated by the loss of ketene, effectively suppressing other sample-specific fragmentation. The correspondence of the product-ion spectra derived from this fragment ion in the current study (namely the unimolecular fragment ion corresponding to $[BH_2-42]^+$ from dGuo-C8-AAF) and that of the BH_2^+ ion obtained previously from the nonacetylated arylamine *N*-(deoxyguanosin-8-yl)-2-aminofluorene, dGuo-C8-AF [11], suggests that loss of ketene from the BH_2^+ fragment of dGuo-C8-AAF does not substantially affect the structure of the aminofluorene or Gua moieties. As detailed below, CID of the BH_2^+ ion derived from dGuo- N^2 -AAF leads to product ions (e.g., water loss) quite different from those observed for the C8 isomer. This difference is attributed to cyclization reactions that may occur prior to fragmentation and interactions between the acetyl group of the carcinogen and the $N1$ or N^2 atom of the Gua base.

Product ions, BH_2^+ from dGuo-C8-AAF. The CID spectrum of the BH_2^+ ion at m/z 373 derived from dGuo-C8-AAF is dominated by just one fragmentation pathway to yield the ion at m/z 331, attributed to ketene loss. A representative spectrum is presented in Figure 2. The spectrum acquired from protonated Gua-C8-

Figure 2. The CID product-ion spectrum of the BH_2^+ ion at m/z 373 obtained by using 100 ng of *N*-(deoxyguanosin-C8-yl)-2-acetylaminofluorene derivatized with MSTFA.

AAF acquired previously by using B/E scans and 8 keV collision energies [9] showed more extensive fragmentation than was observed in this study, perhaps because of the use of higher energy collisions. The fragment ions formed can be rationalized in terms of a Gua-AF intermediate where the loss of ketene proceeds without further altering the structure of the BH_2^+ ion.

We found that the product-ion spectrum of the $[BH_2-42]^+$ at m/z 331 (50 eV) was similar to that reported earlier for protonated Gua-C8-AF obtained by using 8 keV CID [9] and nearly identical when 50 eV CID was used [11] (spectrum not shown). These results suggest that, at least for the C8-substituted species, these ions have the same structures, and thus no other rearrangements are associated with ketene loss. In other words, loss of ketene from protonated, AAF-modified Gua produces a fragment ion that is protonated AF-modified Gua. The fact that such fragmentation seems to occur without any evidence of additional changes in structure is a key point for evaluating further fragmentation from the m/z 331 ion derived from the BH_2^+ ion of dGuo- N^2 -AAF described below.

Product ions, BH_2^+ from dGuo- N^2 -AAF. Collisional activation of the BH_2^+ ion at m/z 373 derived from dGuo- N^2 -AAF yields three major ions (m/z 355, 331, and 314), as shown in Figure 3. The ions observed at m/z 331 and 314 are both attributed to the loss of ketene and, in the case of m/z 314, the loss of ammonia as well. Alternatively, the ion at m/z 314 could arise from protonation of the amide nitrogen and loss of H_2NCOCH_3 . The ion observed at m/z 355 can only be explained by the loss of water from m/z 373, and is of particular interest because such a fragmentation pathway has not been observed in product-ion spectra from nonacetylated C8 [9-11], N^2 -[9] dGuo-arylamines, or acetylated C8-substituted dGuo-arylamide [9, 11] adducts. These observations suggest that although ketene loss from the aglycone may be typical of arylamide-dGuo adducts, the combination of water and ketene loss may be indicative of N^2 -substitution

Figure 3. The CID product-ion spectrum of the BH_2^+ ion at m/z 373 obtained by using 100 ng of 3-(deoxyguanosin- N^2 -yl)-2-acetylaminofluorene derivatized with MSTFA.

of dGuo with arylamides. Moreover, it can be inferred that the acetyl group attached to the exocyclic PAH amino group is involved in some way in the water loss. Other ions formed at lower masses and abundances may be rationalized in terms of fragmentation pathways that proceed through intermediates involving either ketene or water loss and whose formation is discussed in more detail below.

Product ions, m/z 331 from dGuo- N^2 -AAF. Product-ion mass spectra of the m/z 331 ion were obtained because these spectra were expected to be the same as those from an unacetylated N^2 -substituted arylamine-dGuo adduct, dGuo- N^2 -AF. (Although the N^2 -AF adduct has not been reported, the formation of similar adducts in vivo is known to occur frequently when nitro or arylamines are fed to laboratory rodents, albeit in smaller proportions than the C8-substituted adducts [14].) This expected similarity between the structure of the m/z 331 ion and protonated Gua- N^2 -AF is analogous to the results for C8-substituted AAF and AF dGuo adducts discussed in detail above. To our knowledge, no product-ion spectra of any N^2 -arylamines have been acquired under low energy (50 eV) conditions.

The product-ion spectrum of the m/z 331 ion derived from dGuo- N^2 -AAF is presented in Figure 4. This CID spectrum shows more extensive fragmentation than does the spectrum of the related compound, dGuo- N^2 -Aphen, previously acquired by using high energy B/E scans. Only two fragment ions of significant abundance, one resulting from the loss of NH_3 and another from cleavage within the pyrimidine portion of the base, are produced by high energy collisions [9]. The masses of many of the fragment ions shown in Figure 3 are consistent with formation from the Gua- N^2 -AF ion after NH_3 loss. This loss may occur directly if protonation occurs on the arylamine- NH_2 . Otherwise, the abstraction of a hydrogen atom bonded to N1 on the Gua base followed by bond formation between the AF C2 and the Gua N1 may occur. A mechanism for the formation of the m/z 205, 232, and 259 ions is presented in Scheme I. [Bond formation

Figure 4. The CID product-ion spectrum of the deacetylated BH_2^+ ion at m/z 331 obtained by using 100 ng of 3-(deoxyguanosin- N^2 -yl)-2-acetylaminofluorene derivatized with MSTFA.

between the C2 carbon of the PAH and the N^1 position of the Gua (Ia) is assumed but is not required to explain the experimental data.] A subsequent shift in electron density can result in an intermediate configuration from which the ions at m/z 205 (Id), 232 (Ic), and 259 (Ib) can all be produced by a combination of single bond cleavages within the Gua base.

The formation of an ion at m/z 286 may be noted in Figure 4. The fact that the mass of this ion is 45 u removed from the parent ion at m/z 331 suggests that the ion is formed by successive losses of 17 (NH_3) and 28 (CO) u. Another possibility, the loss of 18 (H_2O) and 27 (HCN) u, cannot be conclusively ruled out. However, no $(\text{M} + \text{H} - \text{H}_2\text{O})^+$ or $(\text{M} + \text{H} - \text{HCN})^+$ ions were observed (Figure 4) to suggest such stepwise processes. A likely explanation is that these losses arise

from the keto form of the Gua base (Ia) where only two single bonds ($N^1\text{-C6}$ and C5-C6) need be broken to liberate CO .

Scheme II details the other major ion formation processes that are observed in the CID spectrum of the $(\text{BH}_2\text{-42})^+$ ion (m/z 331 in Figure 4) but that are not delineated in Scheme I. Cleavage of the same two C-N bonds that produced m/z 205 yields the m/z 222 ion (IIa). This ion, which may be diagnostic of arylamine substitution at the N^2 position of Gua, was also reported in the B/E scans of the BH_2^+ ion derived from the related compound dGuo- N^2 -APhen [9]. The m/z 180 ion (IIb) is probably generated directly from the m/z 331 ion by cleavage of the Gua- N^2 to fluorene-C3 bond or from the m/z 222 ion by cleavage of the same Gua- N^2 to fluorene-C3 bond. Of course, loss of ammonia could also precede formation of the m/z 180 ion via fragmentation from the m/z 314 ion (Ia in Scheme I), assuming a ring-opened form and transfer of a proton to the Gua- N^2 atom. Subsequent fragmentation within the aromatic ring itself and loss of HCN would yield the PAH fragment ion at m/z 153.

The ions observed at m/z 135 and 110 in Figure 4 are derived from the Gua portion of the molecule. The ion at m/z 135 corresponds to $(\text{Gua} + \text{H} - \text{NH}_3)^+$. Loss of an exocyclic nitrogen from a nucleoside base has been used to provide evidence for substitution through the exocyclic amine of the base rather than at other locations such as C8 [15, 16]. The ion at m/z 110 was also observed from the N^2 isomer but, as it results from rearrangement within the Gua base, may not be as diagnostic of the position of substitution as the m/z 135 ion.

Product ions, m/z 355 from dGuo- N^2 -AAF. The CID spectra of the isomeric AAF adducts used in this study suggest the hypothesis that ions formed by water and ketene loss may constitute a fragmentation pattern specific for arylamide substitution at the Gua- N^2 posi-

tion. Although these same losses, water and ketene, were reported from the molecular ion in the electron ionization (EI) mass spectrum of the modified base, Gua- N^2 -AAF [16], further investigations using additional N^2 -substituted arylamides will be needed to test fully this hypothesis.

To gain some insight into possible mechanisms of water loss from the BH_2^+ ion at m/z 373 derived from dGuo- N^2 -AAF, product-ion spectra of the source-produced m/z 355 ions were obtained. The product-ion spectrum of m/z 355 is shown in Figure 5. This spectrum is dominated by two ions at m/z 221 and 135, of about equal relative abundance. Both of these ions can be explained by fragmentation from an m/z 355 ion formed via protonation of the acetyl-carbonyl oxygen followed by abstraction of the N^2 -hydrogen and water elimination (IIIa). A possible mechanism for water loss from the m/z 373 BH_2^+ ion and subsequent CID fragmentation of m/z 355 (Figure 5) is proposed in Scheme III. [An analogous structure for the $[M - H_2O]^+$ ion (m/z 354) in the EI mass spectrum of Gua- N^2 -AAF was proposed in ref 16 based on the solution-phase behavior of a similar N^6 -modified adenosine adduct [17].]

The masses and abundances of the product ions from m/z 355 suggest that the two fragments are

Figure 5. The CID product-ion spectrum of the $[BH_2 - H_2O]^+$ ion at m/z 355 obtained by using 100 ng of 3-(deoxyguanosin- N^2 -yl)-2-acetylaminofluorene derivatized with MSTFA.

formed by breaking a specific bond, with charge retention being more or less equally probable on either half of the fragmenting ion. Simple cleavage of the single C2- N^2 bond of the Gua moiety of IIIa can yield either a deaminated Gua ion $[\text{Gua} + \text{H} - \text{NH}_3]^+$ at m/z 135 or a fluorene-derived fragment ion at m/z 221. Both arise from the m/z 355 (IIIa) ion resulting from the water-loss mechanism described above, depending upon which fragment retains the charge-bearing proton. Because there are similar functional groups within

the two fragments (both are heterocyclic amines), the charge-bearing proton could easily reside on either fragment. It should also be pointed out that from the original structure (i.e., before water loss), an ion at m/z 135 can result directly from a straightforward loss of the carcinogen and N^2 (exocyclic) amine, yielding the deaminated Gua ion. Thus, the fragment at m/z 135 in the CID spectrum of dGuo- N^2 -AAF can arise with or without a prior loss of water. As pointed out earlier, an ion at m/z 135, $[\text{Gua} + \text{H} - \text{NH}_3]^+$ was associated with N^2 -substitution of guanine in the EI spectrum obtained from dG- N^2 -AAF [16]. That this fragment was observed even after water loss is evidence that water loss involves the acetyl-oxygen rather than the guanyl-oxygen.

Of lesser relative abundance in the CID spectrum in Figure 5 are the ions observed at m/z 180 and 153. The low abundance of the m/z 180 ion from the CID spectrum of the m/z 355 ion relative to that of the m/z 331 ion would be in accord with the difficulty of forming an ion like IIb from the cyclic structure IIIa postulated in Scheme III. (A three-membered ring species is suggested in Scheme II, but an isomeric carbonium-ion structure may also be a reasonable alternative.) As was previously proposed from CID of m/z 331, the ion observed at m/z 153 could be formed by HCN loss from the m/z 180 ion. The fact that both the m/z 180 and 153 ions are observed together in two different product-ion spectra supports the notion that the m/z 153 ion is formed from the m/z 180 ion.

Conclusion

Product-ion studies of the BH_2^+ and other significant source-produced ions derived from the isomeric 2-acetylaminofluorene adducts of dGuo were used to differentiate dGuo- N^2 -AAF from the isomeric adduct dGuo-C8-AAF and to provide evidence for ion formation processes that appear to be mediated by the acetyl side chain. The loss of ketene as a product ion from the aglycone appears to be typical of arylamide adducts, whereas loss of both ketene and water may be specific for N^2 -substitution of the arylamide on Gua. The product-ion spectra of the BH_2^+ ion from dGuo-C8-AAF showed almost exclusive fragmentation due to the loss of ketene. The loss of H_2O from the BH_2^+ ion of the dGuo- N^2 -AAF adduct may be explained by protonation of the carbonyl oxygen within the acetyl group followed by water elimination and the bonding of the acetyl carbonyl carbon to the N^2 position of the Gua

base. This would be in accord with previous interpretations of water loss in the EI mass spectrum of Gua- N^2 -AAF [16]. The product-ion spectra of the protonated Gua- N^2 -AF ion suggest that much of the fragmentation occurs after NH_3 loss, perhaps with bond formation between the C2 of the aminofluorene and the N^1 position on Gua. The lack of fragmentation within the fluorene (carcinogen) moiety, as seen in the product-ion spectra of the N^2 -substituted AAF and AF adducts of dGuo in this study, is not unlike the behavior observed from C8-substituted arylamine adducts previously studied, which also did not show cleavage within the polycyclic carcinogen group. This suggests that these ion formation processes, resulting primarily from cleavages within the nucleoside-base, may be common to other dGuo- N^2 -arylamine and arylamide adducts.

Acknowledgments

We thank the reviewers for their comments regarding likely fragmentation pathways, particularly the water-loss mechanism shown in Scheme III. This work was supported in part by the Center for Indoor Air Research (CIAR #90-002).

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