Mass Spectrometric Methods for Distinguishing Structural Isomers of Glutathione Conjugates of Estrone and Estradiol*

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Collisionally activated decompositions (CAD) of $[M+H]^+$ ions from two sets (estrone and estradiol) of three isomeric glutathione (GSH) conjugates were studied by using five tandem mass spectrometric methods: (1) low energy (LE) CAD in an ion trap, (2) LE CAD in a triple quadrupole, (3) electrospray ionization (ESI)-source CAD in a tandem four sector, (4) high energy (HE) CAD of both ESI-produced and fast-atom bombardment (FAB)-produced ions in a tandem four-sector mass spectrometer, and (5) metastable-ion decompositions of FAB-produced ions. Four types of fragment ions are produced. The first type, formed from cleavage of the peptide backbone, gives rise to modified b_2 , modified y_2 , y_2 , and b_1 ions. These fragments are observed with all the methods and show that the catechol estrogen attachment is at the cysteine moiety of the GSH. Internal fragment ions are the second type, and they also support that the modification is at cysteine. The third type involves fragmentation of the C-S bond to give an ion containing the steroid bonded to the sulfur. The fourth type of fragment ion is similar to the third but involves oxidation of the steroid ring and reduction of the GSH moiety; it is the most isomer specific of the four. The isomer-specific ions are of relatively low abundance in the product-ion spectra taken on the triple quadrupole and ion trap, but their abundances can be improved by increasing the collision energy. ESI source-CAD and the HE-CAD spectra of the isomers are the most distinctive because abundant product ions of all four types are seen in a single spectrum. (J Am Soc Mass Spectrom 1998, 9, 612-619) © 1998 American Society for Mass Spectrometry

Endogenous steroid hormones, estrone (E_1) and 17β -estradiol (E_2), are metabolized via two major pathways: 2- and 4-hydroxylation to form catechol estrogens (CE) and 16α -hydroxylation [1, 2]. The CE metabolites are usually methylated by catechol-O-methyltransferases (COMT) or conjugated to form glucuronides and sulfates. All these pathways serve to prevent oxidation of CE to reactive electrophilic quinones. When the level of CE is elevated and/or the activity of conjugating enzymes is decreased, CE accumulate and can be further oxidized by peroxidases or cytochrome P450 to form CE quinones. The quinones

are electrophilic and are susceptible to adduction, via Michael addition, to the nucleophilic groups of cellular macromolecules [3-5] and glutathione (GSH) [6]. Binding of CE quinones to DNA can lead to carcinogenesis [5], whereas binding to GSH leads to excretion [6].

Adduction to the tripeptide glutathione (γ -glutamyl-L-cystenylglycine), which occurs in all living cells [6], and most abundantly in the liver cells, is one of the major excretory pathways for many structurally diverse substrates ranging from xenobiotics [7] to toxic metals [8]. In most cells, the GSH concentration is in the range of 0.1 to 10 mM, and the body maintains the ratio between the oxidized (GS-SG) and the reduced (GSH) forms as low as 0.1 [9], facilitating the conjugation of the reduced GSH by glutathione-S-transferases (GST) to exogenous and endogenous electrophiles [10].

Conjugation to GSH and adduction to DNA involve

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similar chemistry. Because adduct formation is a required early step in tumor initiation, the detection and characterization of estrogen-DNA adducts in target tissues is important for understanding carcinogenesis and for estimating risk. But detection and characterization of an estrogen-DNA adduct are difficult because only one base in 10^6 – 10^8 is modified. On the other hand, quinones readily react with GSH to give more abundant and easily measured adducts. Mass spectrometry, in combination with tandem mass spectrometry, has been used to determine a variety of GSH conjugates [11–14], including those from *in vitro* and *in vivo* experiments [15]; a preliminary announcement of this work was made [16].

In the research reported in this article, we have taken the challenge of determining isomeric GSH adducts of estrone and estradiol. We compare the results from four tandem mass spectrometric methods in which MS/MS experiments only were conducted and from ESI-source CAD. The outcome of the first two methods is an LE-CAD spectrum of product ions from ESI-produced $[M+H]^+$ ions; the product ions were formed in either an ion trap (method 1) or a triple-quadrupole mass spectrometer (method 2). Method 3 gives ESI-source CAD spectra, and method 4 produces an HE CAD spectrum of either ESI or FAB-produced ions; methods 3 and 4 utilize a tandem four-sector mass spectrometer. The outcome of method 5 is a metastable-ion (MI) spectrum from the four-sector instrument. The focus of the article is the evaluation of mass spectrometric methods for distinguishing structural isomers of catechol estrogen-GSH (CE-GSH) conjugates; we chose not to evaluate other analytical issues (limits of detection and quantification, linearity of calibration, etc.) even though we were able to obtain product-ion spectra, in some cases, of femtomole quantities of adducts.

Experimental

Materials

The conjugates were prepared by mixing the corresponding quinones in acetonitrile with a 50/50 water/acetic acid solution of GSH [17]. UV, NMR, exact-mass measurements, and tandem mass spectrometry (MS/MS) were used to confirm the structures of the GSH conjugates of estrone and estradiol. Synthesis and confirmation of these conjugates were described in a separate article [17].

Mass Spectrometry

The first set of LE-CAD spectra were acquired by using a Finnigan (Finnigan MAT, San Jose, CA, USA) quadrupole ion trap (LCQ) equipped with a Finnigan ESI source. Samples were dissolved in 50/49/1 methanol/water/acetic acid to give a final concentration of 5 pmol/ μ L and were introduced via a 5- μ L loop injector into a 10- μ L/min flow of 50/49/1 methanol/water/

acetic acid. Although the detection limit for modified GSH conjugates was approximately 25 fmol, a higher concentration was used to insure that the tandem mass spectra had a good signal-to-noise ratio. The spray needle was held at 4.2 kV, and 4.8×10^5 Pa coaxial flow of nitrogen was used to stabilize the spray. A heated (200 °C) stainless steel capillary held at 5 V served as a counter electrode. Helium was introduced to a pressure of 1 mtorr for improving the trapping efficiency of the ion trap (background pressure of 2.7×10^{-3} Pa) and for use as the collision gas during the CAD event. Ions were detected by a 15-kV conversion dynode interfaced to a channeltron electron multiplier.

The second set of LE-CAD experiments was carried out with a Finnigan TSQ 7000 triple quadrupole mass spectrometer, which was equipped with a Finnigan ESI source similar to that of the LCQ. The electrospray needle was held at 5000 V, and the counter electrode, a heated stainless steel capillary, was held at 5 V. Samples were dissolved in 50/49/1 methanol/water/acetic acid to give a final concentration of 50 pmol/ μ L and were loop injected into a 5- μ L/min flow of the same solvent. The detection limit for steroid-modified GSH with the triple quadrupole is in the range of 5 to 50 fmol, but a higher concentration was used so that high quality tandem mass spectra could be obtained. For all MS/MS experiments, argon was used as collision gas at a pressure of approximately 0.33 Pa. For obtaining the breakdown graphs, the collision energy was varied from 20 to 60 eV by changing the offset voltage of Q2 with respect to that of Q1. For comparison of product-ion spectra, a collision energy of 25 eV was chosen.

ESI source-CAD, HE-CAD, and MI spectra of product ions were obtained by using a VG ZAB-T (Manchester, UK) four-sector tandem mass spectrometer [18] equipped with a VG ESI source. The ESI needle was at 8000 V and the counter electrode ("pepper pot") at 5000 V. To induce CAD in the high-pressure region between the sampling cone and the skimmer, the sampling cone potential was increased to approximately 135 V above the optimal operating potential of 4177 V. Skimmer lens, skimmer, ring electrode, and hexapole were typically held at 4125, 4120, 4115, and 4115 V, respectively. Nitrogen was used as bath and nebulizer gas with a flow rate of 400 and 12 L/h, respectively. Samples (50 pmol/ μ L), dissolved in 50/49/1 methanol/water/acetic acid, were injected into the flow of a similar solvent system via a six port Rheodyne 7125 injector with a 20- μ L external loop. A Harvard model 22 syringe pump was used to infuse the solvent at a flow rate of 10 μ L/min.

For FAB, samples were dissolved in 50/50 water/methanol to give a final concentration of 2 nmol/ μ L. A 1- μ L aliquot was loaded on the probe along with 1 μ L of glycerol with 1% trifluoroacetic acid (GLY/TFA). A Cs^+ ion gun operated at 30 kV was used to desorb the ions, which were accelerated to +8.0 keV.

HE CAD mass spectra of ESI-produced ions were obtained after precursor-ion activation in the third

field-free region (between MS1 and MS2). Sufficient helium was added to the collision cell, which was floated at 2 kV, to attenuate the ion beam by 80%. The first (MS1) and second-stage (MS2) mass spectrometers were operated at resolving powers of 1000 and 800 (full width at half-height definition), respectively. To obtain high-energy CAD spectra of FAB-produced ions, the collision cell was floated at 4 kV, and sufficient helium was introduced into the cell to attenuate the ion beam by 50%. Ten to fifteen 15-s scans were signal-averaged for each spectrum. The metastable-ion decompositions of FAB-produced ions were followed under the same conditions as the HE CAD experiments, but no collision gas was introduced into the collision cell. All MI and CAD spectra were acquired by using a VG (Manchester, UK) data system equipped with a DEC Alpha 3000 work station, OPUS V 3.1X software, and a VG SIOS I interface.

Nomenclature

When referring to both E_1 and E_2 catechols, we will use the term CE. The terms E_1 -GSH and E_2 -GSH are used to refer to all isomers of estrone and estradiol, respectively. The nomenclature proposed by Roepstorff and Fohlman, as modified by Biemann [19], was used to describe the peptide backbone fragmentation of the GSH moiety. All GSH fragment ions containing catechol estrogen moiety, R_x , are referred to as modified

Figure 2. Tandem mass spectra of ESI-produced $[M+H]^+$ ions from (A) 4-OHE₂-2-SG, (B) 2-OHE₂-4-SG, and (C) 2-OHE₂-1-SG, obtained by using an ion-trap mass spectrometer.

backbone fragment ions. Internal fragment ions are described by the nomenclature of Gaskell and co-workers [20].

Results and Discussion

In this study, we evaluate the effectiveness of five tandem mass spectrometric methods to characterize two sets of isomeric CE-modified GSH conjugates. One set consists of three isomers of estrone conjugates, and the second is represented by three isomers of estradiol conjugates (see Figures 1 and 2 for structures and abbreviations).

Low-Energy CAD Spectra of ESI-Produced Ions on an Ion Trap

To establish a reference point, we discuss briefly the LE CAD of GSH (γ -glu-cys-gly) itself. ESI of GSH gives $[M+H]^+$ and $[M+Na]^+$ ions of m/z 308 and 330, respectively. LE CAD of the $[M+H]^+$ ions gives the expected z_2 , y_2 , and b_2 ions of m/z 162, 179, and 233.

LE CAD product-ion spectra of the $[M+H]^+$ ions (m/z 592) of catechol E_1 -GSH conjugates 4-OHE₁-2-SG, 2-OHE₁-4-SG, and 2-OHE₁-1-SG are nearly identical (Figure 1). Similarly, the product-ion spectra of catechol- E_2 -GSH $[M+H]^+$ ions of m/z 594 from 4-OHE₂-2-SG, 2-OHE₂-4-SG, and 2-OHE₂-1-SG are also very similar (Figure 2). Peptide backbone fragmentations give rise to modified b_2 , and y_2 ions, which we designate as

Figure 1. Tandem mass spectra of ESI-produced $[M+H]^+$ ions from (A) 4-OHE₁-2-SG, (B) 2-OHE₁-4-SG, and (C) 2-OHE₁-1-SG, obtained by using an ion-trap mass spectrometer.

Scheme I. Four types of fragment ions observed with CE-GSH conjugates.

b_2 and y_2 , suggesting that the Cys moiety has been modified. We will refer to these and other product ions from backbone cleavages as “type 1” (see Scheme I). For E_1 and E_2 adducts, these fragment ions are at m/z 517 and 463 (Figure 1) and 519 and 465 (Figure 2), respectively, and are consistent with those reported in earlier studies of the LE CAD of GSH adducts [11–14]. Because there is a low-mass cutoff for the ion trap, the complementary b_1 and y_1 fragment ions could not be observed.

Internal fragment ions of the structure $^+NH_2=CHCH_2S-R_x$ (Schemes I and II), where R_x is either the estrone or estradiol moiety are at m/z 360 (Figure 1) and 362 (Figure 2). These fragment ions are common in tandem mass spectra of GSH conjugates and are referred to as *g* ions, following the nomenclature proposed by Gaskell and co-workers [20]. The same group also suggested that the *h* fragment, $^+CH=CH-S-R_x$, is formed by loss of ammonia from the *g* fragment ion. For the purpose of organizing this article, we will call these ions “type 2.”

For both sets of conjugates, the fragment ion of m/z 317 (E_1) or 319 (E_2) from the cleavage of the C–S bond is the dominant product ion (Schemes I and III). This

Scheme II. Proposed mechanism for formation of internal fragment ions.

Scheme III. Proposed mechanism for formation of ion of m/z 317 and 299 and 319 and 301 from GSH conjugates of E_1 and E_2 , respectively.

fragmentation releases the steroid as an ion with the sulfur attached. The m/z 317 and 319 ions subsequently lose H₂O to give the product ions of m/z 299 or 301, respectively. These and the ions discussed next will be called “type 3.”

The product ion of m/z 284 from E_1 -GSH conjugates and 286 from E_2 -GSH conjugates is likely to result from loss of an HS radical from the ions of m/z 317 or 319, respectively. This loss of HS is analogous to that from alkyl phenyl thioethers [21]. Loss of H₂S from GSH adducts was also observed previously [22] and may account for the low-abundance product ions of m/z 283 and 285 from the m/z 317 (E_1) and 319 (E_2) ions, respectively.

An interesting pair of ions are those of m/z 315 and 297 from the E_1 -GSH conjugates and 317 and 299 from the E_2 -GSH conjugates; we will refer to these as “type 4.” These product ions are not detectable when the GSH is attached at position 2 of the A ring of the steroid, barely detectable when the attachment is at position 4, and definitely detectable when the attachment is at position 1. Although neither fragment is of high abundance in the ion-trap, product-ion spectra, they assume considerably more importance in ESI-source CAD and HE CAD spectra, which will be discussed later.

Although the ion-trap spectra strongly suggest that Cys is modified by the steroid, the inability to observe b_1 , y_1 in an MS/MS experiment and the relatively low abundances of the y_2 and type-4 fragment ions make the identification of isomeric GSH conjugates difficult when using only one stage of MS/MS.

CAD of ESI-Produced Ions in a Triple Quadrupole

To evaluate triple quadrupole mass spectrometers for distinguishing these structural isomers and to assess if there is additional information for distinguishing isomers in the lower mass region of the tandem mass

Figure 3. Breakdown graph of ESI-produced $[M+H]^+$ ions from (A) 4-OHE₂-2-SG and (B) 2-OHE₂-1-SG, obtained using a triple-quadrupole mass spectrometer.

spectra, we investigated the same set of conjugates with this instrumentation.

Energy-resolved CAD spectra. Energy-resolved spectra were obtained by systematically varying the collision energy and obtaining product-ion abundances as a function of that energy. Breakdown graphs for 4-OHE₂-2-SG (Figure 3A) and 2-OHE₂-1-SG (Figure 3B) show that the y_2 of m/z 465 and the m/z 519 and 319 ions are primary fragments. With increasing collision energy in the range of 10–45 eV, the ion of m/z 465 fragments in part to form the ion of m/z 319. Increasing the collision energy above 35 eV causes the abundances of the isomer-specific fragment ions (m/z 317, 299, and 286) and the low-mass fragment ions from the GSH moiety (m/z 130, 179, 177, 205, and 207) to grow at the expense of the higher mass-to-charge ratio (>345) fragment ions (some of these fragments are not shown in the figure to minimize congestion). The breakdown graphs clearly show isomeric differences; for 2-OHE₂-1-SG, the m/z 317 and 299 (type-4 ions) become the important second- and third-generation products, whereas they are not detectable for 4-OHE₂-2-SG.

The breakdown graphs clearly show that the choice of collision energy is critical for distinguishing the spectra of the isomers. Because the four types of fragment ions can be seen at 25 eV, we chose that energy to compare tandem mass spectra of the six isomers.

LE CAD of CE-GSH adducts. At 25-eV collision energy, both sets of three isomers fragment most readily by C–S bond cleavage (spectra not given). The peptide-backbone fragmentation gives the y_2 and b_2 fragment ions at m/z 517 and 463, respectively. The type-2 product ions of m/z 360 and 343, which are $^+NH_2=$

$CHCH_2S-R_x$ and $^+CH=CH-S-R_x$, respectively, are relatively less abundant in the spectrum of 4-OHE₁-2-SG, than in the spectra of the other two isomers. This trend also follows that obtained when using the ion trap.

The product ions of m/z 317 (E_1 adducts) or 319 (E_2 adducts) are most abundant. Differences pertain for the m/z 315 ion, which is absent in the spectrum of 4-OHE₁-2-SG, of modest abundance in that of 2-OHE₁-4-SG, and relatively more abundant in that of 2-OHE₁-1-SG. The fragment ion of m/z 297, formed from loss of a water molecule from the ion of m/z 315, is absent in the spectra of 4-OHE₁-2-SG and present at modest and high abundance in the spectra of 2-OHE₁-4-SG and 2-OHE₁-1-SG, respectively. An analogous situation applies to the m/z 317 and 299 ion from the E_2 isomers. These two pairs of product ions (i.e., of m/z 315/297 and 317/299) are most important for distinguishing isomers.

Fragment ions of m/z 284 formed by loss of an HS radical from the m/z 317 ion, are also isomer specific. They are readily detectable from 4-OHE₁-2-SG, modestly abundant from 2-OHE₁-4-SG, and barely detectable from 2-OHE₁-1-SG, an order that is opposite those for the m/z 315 and 297 ions. The corresponding fragments for the estradiol conjugates shift upward by 2 u, but the trends for dissociation are similar to those for the E_1 conjugates. Although we do not understand the reason for these trends, we note that the breakdown curves (Figure 3) show that the m/z 286 ion is the important terminal fragment for 4-OHE₂-2-SG, whereas the m/z 299 ion plays the same role for 2-OHE₂-1-SG.

Some low-mass fragment ions are also useful for distinguishing the isomers, but it is difficult to rationalize the trends. The unmodified b_1 ion of m/z 130 is one example (it is likely that this ion is a fragment of $^+b_1$).

Figure 4. ESI-source CAD of ions from (A) 4-OHE₁-2-SG, (B) 2-OHE₁-4-SG, and (C) 2-OHE₁-1-SG, obtained by using a tandem four-sector mass spectrometer.

Loss of 129 u from GSH conjugates is one process that has been used in constant-neutral loss scanning to detect GSH conjugates from in vivo experiments [15].

Although establishing the expected attachment of CE to Cys is relatively simple using low-mass product ions, assigning a spectrum to a specific isomer is difficult. Results with the triple quadrupole, which are similar to those obtained with the ion trap, provide some evidence to distinguish the isomers. For example, although the fragment ions of m/z 315/317 and 297/299 are not clear evidence in the 25-eV spectra of 2-CE-4-SG, increasing the collision energy enhances the abundance of these fragments but at the expense of the high-mass fragments. Clearly an integrated view of the energy-dependent fragmentation is needed to distinguish the isomers because no single LE CAD can provide a complete identification. The breakdown curves serve that function, but they require time and sample to generate. These limitations of LE CAD spectra prompted us to study these adducts by using ESI-source CAD and HE CAD on a tandem four-sector mass spectrometer.

ESI-Source CAD in a Sector Mass Spectrometer

Although ESI-source CAD requires introduction of pure samples into the mass spectrometer, it can have, when conducted on a magnetic sector mass spectrometer, relatively high-mass resolving power. ESI of E₁ conjugates yields dominant $[M+H]^+$ (m/z 592) and negligible $[M+Na]^+$ (m/z 614). Similarly, ESI of E₂

Figure 5. Tandem mass spectra of ESI-produced $[M+H]^+$ ions from (A) 4-OHE₁-2-SG, (B) 2-OHE₁-4-SG, and (C) 2-OHE₁-1-SG, obtained by using a tandem four-sector mass spectrometer.

conjugates also yields abundant $[M+H]^+$ and some $[M+Na]^+$. Increasing the sampling cone potential by 135 V above the optimal value causes dissociation in the high-pressure region between the sampling cone and the skimmer, giving spectra (Figure 4) that are similar to those from the ion trap and the triple quadrupole except the isomerically distinctive m/z 284 and type-4 ions are more apparent. The type-4 fragments are barely detectable in the spectra of 4-OHE₁-2-SG and 4-OHE₂-2-SG, whereas for 2-OHE₁-4-SG and 2-OHE₂-4-SG, they are quite abundant and even more abundant for 2-OHE₁-1-SG and 2-OHE₂-1-SG. Some key low-mass fragments, b₁, a₂, and y₂ ions, are present in all spectra, but they are of low abundance. The unmodified a₂ and y₂ ions are presumably products of the corresponding modified species. Nevertheless, unambiguous assignments of the three E₁ and E₂ isomers are possible with the mass spectra from this method.

High-Energy CAD Spectra

The success in distinguishing isomers achieved by using ESI-source CAD provided additional incentive to test HE CAD for distinguishing the isomeric GSH adducts of E₁ and E₂. We chose to produce the precursor ions by both ESI and FAB.

ESI-produced ions. In contrast to LE-CAD spectra, abundant isomer-specific ions (type 4 and m/z 284/286) as well as types 1–3 fragment ions are observed in HE CAD spectra (Figure 5). The important distinguish-

Figure 6. Tandem mass spectra of ESI-produced $[M+H]^+$ ions from (A) 4-OHE₂-2-SG, (B) 2-OHE₂-4-SG, and (C) 2-OHE₂-1-SG, obtained by using a tandem four-sector mass spectrometer.

ing features for 4-OHE₁-2-SG with respect to the other isomers are the negligible abundances of the m/z 315 and 297 ions and the abundant m/z 284 ion. In the spectrum of 2-OHE₁-4-SG, the m/z 315 and 297 ions make their appearance at modest abundance. For the third isomer, 2-OHE₁-1-SG, we see the highest abundances for the m/z 315 and 297 ions and the lowest abundances of the m/z 284 ion and the internal g ion of m/z 360.

HE-CAD spectra from E₂ conjugates, shown in Figure 6 are similar to those of E₁ conjugates. All fragments containing the steroid are observed, but their masses are shifted by 2 u. Recalling that the key fragments for distinguishing the isomers are of type 4 (m/z 317 and 299), and the m/z 286 ion, we note that the ions of m/z 317 and 299 are most abundant in the spectra of 2-OHE₂-1-SG, not detectable in that of 4-OHE₂-2-SG, and intermediate in that of the 2-OHE₂-4-SG; this trend as the same as that for the E₁ conjugates.

We propose as a mechanism for the type-4 ions that oxidation of the steroid moiety occurs, involving transfer of a favorable tertiary hydrogen (Schemes I and IV) for 2-OHE₁-1-SG and 2-OHE₂-1-SG. For 2-OHE₁-4-SG and 2-OHE₂-4-SG adducts, oxidation of the steroid moiety involves transfer of a secondary hydrogen (Scheme V), diminishing the abundances of the m/z 315 (from E₁) and 317 (from E₂) ions. With 4-OHE₁-2-SG and 4-OHE₂-2-SG adducts, oxidation of the steroid is difficult because the sulfur attachment site is now more remote from the hydrogen sources. The proposed origin

Scheme IV. Proposed mechanism for the formation of ion of m/z 315 (E₁) and 317 (E₂) from 2-OHE₁-1-SG conjugates.

of the oxidized-steroid ion [m/z 517 (E₁) and 519 (E₂)] is consistent with the breakdown curves (Figure 3).

FAB-produced ions. FAB-produced $[M+H]^+$ ions of glutathione and of the isomeric conjugates were studied by using HE CAD to learn about the fragmentation of these ions under conditions in which the ions have higher internal energy [23]. Product ions from backbone fragmentation (type 1) become more pronounced because the proton can now attach to low proton-affinity amide nitrogens. This makes FAB in combination with HE CAD, the method of choice for obtaining sequence information of peptides [24], although its sensitivity is not high.

The fragmentation behavior of the unmodified GSH, under high-energy conditions, is different from that observed under LE CAD conditions. The ion of m/z 76 (y_1), 84 ($a_1 - H_2O$), 179 (y_2) and 130 (b_1) are more abundant upon high-energy collisions. The z_2 (of m/z 162) and b_2 (of m/z 233) are less abundant than upon low-energy activation. The HE CAD spectrum of ESI-produced ions is similar to that of FAB-produced ions.

HE CAD spectra of FAB-produced E₁ and E₂-conju-

Scheme V. Proposed mechanism for the formation of ion of m/z 315 (E₁) and 317 (E₂) from 2-OHE₁-4-SG conjugates.

gate ions show clearly the trend established for ESI-produced ions: the m/z 315 (from E_1) and 317 (from E_2) ions and the water-loss products at m/z 297 and 299 are absent, modest, and high abundance in the spectra of 4-OHE-2-SG, 2-OHE-4-SG, and 2-OHE-1-SG, respectively. In addition, the low-mass region of the spectra shows that more abundant backbone-cleavage (type 1) product ions are formed.

Metastable-Ion Spectra

Ions with relatively higher internal energy than the stable ions fragment during transit to the detector and give metastable-ion spectra. Similar to the breakdown graphs obtained using the triple quadrupole, MI spectra give information on the lowest energy fragments, which are usually first- and second-generation product ions. The ions are less abundant than in CAD spectra, explaining the need for a magnification factor of 10 or 20 times greater than that used for CAD spectra.

A typical MI spectrum contains, as the most abundant product ion, that of m/z 463, which is a y_2 ion formed from the $[M+H]^+$ ions, showing that this first-generation ion is formed by the lowest energy process. Other fragment ions at m/z 575 ($[M+H - NH_3]^+$), 574 ($[M+H - H_2O]^+$), 173 (d_2), and 130 (b_1) have relative abundances between 5% and 30%. These MI spectra have little utility for structure determination.

Conclusions

A long-standing goal of our research has been to develop an understanding of the fragmentation chemistry of carcinogen-DNA adducts to assist tumor-initiation studies. Isomer distinction is important in reaching our goal because some isomeric adducts depurinate, some produce lesions, and others are harmless. Detecting DNA modifications to estimate risk is difficult because levels of modification are small. Therefore, we are expanding our research to GSH conjugates, which, when bound to estrogens, may be particularly important for estimating risk for breast cancer.

Unambiguous identification of the spectra relies on the type-4 ions of m/z 315 and 297 for E_1 conjugates and of m/z 317 and 299 for E_2 conjugates. The formation of these ions is most favored when the GSH attachment is at position 1 of the steroid, but still occurs when the attachment is at position 4. We picture these unusual isomerically distinctive reactions as proceeding by oxidation of the steroid moiety and reduction of the departing neutral GSH. These trends are more obvious in HE CAD and in ESI-source CAD spectra than in the LE CAD spectra from ion-trap and triple-quadrupole tandem instruments, although higher order MS/MS experiments with the ion trap may produce the desired information. Increasing the collision energy in the low-energy regime increases the relative abundances of these ions at the expense of high-mass ions. LE CAD

requires a tradeoff when choosing the collision energy, and an uninformed choice without a reference compound may give incomplete information.

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