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Fragmentation of protonated dansyl-labeled amines for structural analysis of amine-containing metabolites

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This work is dedicated to Professor Alex Harrison on the occasion of his 80th birthday and in recognition of his important contributions to ion chemistry and mass spectrometry.

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ABSTRACT

Dansylation of amine-containing metabolites has been shown to provide a significant enhancement in detectability, thereby allowing the generation of a more comprehensive metabolome profile of biofluids using liquid chromatography (LC) electrospray ionization (ESI) mass spectrometry (MS). The relatively stable structures of dansyl-labeled amines afford no fragmentation during the ESI and ion transport to a mass analyzer, ensuring that all peaks detected are from the metabolites, not fragment ions produced, for example, in the interface region. However, for deducing or confirming chemical structures of metabolites, generation of fragment ions of the intact molecular ions by tandem MS is required. We report a study of the fragmentation behaviors of protonated dansyl-labeled amines in comparison to those of the unlabeled counterparts. Characteristic fragment ions of unlabeled amines were observed in a quadrupole linear trap (QTrap) tandem mass spectrometer, while collision-induced dissociation (CID) of the corresponding labeled amine ions mainly produced the fragment ions that contain the dansyl moiety with neutral losses of parts of the original amine molecules. In most cases, no fragment ions from the original amine molecules were detected from a labeled amine. MS^3 in the linear trap did not generate any useful second generation of fragment ions from the original amine molecules. However, it was found that by increasing the skimmer voltage to produce the fragment ions of the labeled amine in the skimmer region, followed by a priori selection of the fragment ion in the first quadrupole mass analyzer with an m/z value corresponding to the mass difference between the molecular ion of the dansyl-labeled amine and the expected mass of the dansyl group, second generation of fragment ions could be produced by CID and stored in the linear trap for improved detection. These second generation of fragment ions generally show a similar fragmentation pattern to that of the protonated unlabeled amine. In some cases, additional fragment ions were found. Thus, chemical structure information of dansyl-labeled amines could be generated using MS/MS and this pseudo-MS^3 approach.

1. Introduction

Liquid chromatography (LC)–mass spectrometry (MS) has been increasingly used for metabolome profiling as it is highly sensitive and specific [1–3]. However, due to the great diversity of physiochemical properties of metabolites, it is difficult to detect and identify all the metabolites with the LC–MS-based metabolome analysis. One approach to meeting this challenge is to classify or fractionate all the metabolites into different groups according to their functional groups, followed by targeted analysis of the individual groups of metabolites using LC–MS.

Our group has been developing a metabolome profiling platform whereby a metabolome sample is selectively labeled with an isotope reagent that reacts with a specific functional group. The labeling reagents are rationally designed to improve the performance of LC separation (i.e., better retention on a high-efficiency reversed-phase column) and electrospray ionization (i.e., better detectability and higher sensitivity in overall detection). We reported the $^{13}C_2$- and $^{12}C_2$-dansylation chemistry for profiling amine- and phenol-containing metabolites [4] and $^{13}C_2$- and $^{12}C_2$-p-dimethylaminophenacyl (DmPA) bromide chemistry for profiling carboxylic acid-containing metabolites [5]. Our work on the use of $^{14}N_2$-/$^{15}N_2$-dansylhydrazine for profiling ketones, aldehydes, and sugars has been submitted for publication. These three isotope labeling chemistries offer a convenient and quantitative route to profiling a large number of metabolites; more than 80% of the 8000 known human metabolites in Human Metabolome Database or HMDB [6] contain one or more of the targeted functional groups. As an example, using a two-dimensional LC–MS system, more than 3500 putative amine- and phenol-containing metabolites could be detected from a human urine sample [7]. The labeled metabolites show significant improvement in

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detectability over their unlabeled counterparts (i.e., 10–1000 fold signal enhancement can be obtained by dansylation).

While rational design of chemical labeling can improve the overall detection of the metabolome, it is also critical to consider another important aspect of the metabolome profiling work, i.e., metabolite identification. With chemical labeling, particularly using a large molecular tag to affect the physiochemical properties of unlabeled metabolites so to improve their chromatography retention, enhance ionization efficiency, and reduce low-mass background interference in ESI, one important question that needs to be addressed is: can the labeled metabolites produce useful fragment ions during the tandem MS analysis for structural analysis?

In this work, we report a study of ESI-MS/MS fragmentation pathways of 32 amine-containing metabolites and compare their fragmentation patterns before and after dansylation labeling. We illustrate that MS/MS analysis of the fragment ions produced in the skimmer region from the protonated dansyl amine with the m/z value corresponding to the protonated unlabeled amine can generate similar fragmentation patterns to those of the unlabeled metabolites, suggesting that structural information can be obtained from tandem MS analysis of the dansylated compounds.

2. Experimental

2.1. Chemical and reagents

All chemicals and reagents were purchased from Sigma–Aldrich Canada (Markham, ON, Canada) except those otherwise noted. LC–MS grade water and acetonitrile (ACN) were purchased from Thermo Fisher Scientific (Edmonton, AB, Canada).

2.2. Labeling reaction

The synthesis of ^{13}C-dansyl chloride as the isotope labeling reagent has been described by Guo and Li [4]. The dansylation labeling reaction has also been described [4], but with some minor changes. Briefly, amine standard compounds were dissolved with ACN/H_2O (50:50) at a concentration of 100 μM. Fifty microliters of standard solutions were mixed with 25 μL sodium carbonate/sodium bicarbonate buffer (500 mM, pH 9.4) and 25 μL ACN in reaction vials. ^{12}C-dansyl chloride solution in ACN (18 mg/mL) or ^{13}C-dansyl chloride solution in ACN (18 mg/mL) was then added, and the reaction stood for 1 h at 60 °C. After 60 min, 10 μL NaOH (250 mM) was added to the reaction mixture to consume the excess dansyl chloride and quench the labeling reaction. After additional 10 min incubation at 60 °C, 50 μL of formic acid in ACN/H_2O (425 mM) was added to neutralize the solution. Finally, the dansylation labeled solutions were diluted 5 folds with ACN/H_2O (10:90) containing 0.1% formic acid for MS analysis.

2.3. Direct flow injection-MS/MS

An AB Sciex 2000 QTrap LC–MS/MS system (AB Sciex, Toronto) was used. The sample solutions were infused directly by a syringe pump at a flow rate of 5 μL/min. The MS instrument was operated under the following conditions: Curtain Gas (CUR) 15 psi, IonSpray Voltage (IS) 4800 V, Temperature (TEM) 250 °C, Ion Source Gas 1 (GS1) 20 psi, Ion Source Gas 2 (GS2) 15 psi. The mass range was set at m/z 50–1000. An enhanced MS (EMS) scan was performed first to find the protonated molecular ion, followed by an enhanced product ion (EPI) scan to generate an MS/MS spectrum. EPI scans were also carried out to generate MS^3 spectra of the high intensity fragment ions observed in MS/MS spectrum. Declustering potential (DP) and collision energy (CE) were adjusted for each different scan. To generate the pseudo-MS^3 spectra from the labeled amines, the fragment ions were first produced in the skimmer region by raising DP to 45 V, followed by selecting the skimmer-fragment ions using the first quadrupole mass analyzer (Q1) that were then subjected to collision-induced dissociation (CID) in Q2. The second generation product ions were analyzed by EPI scan in the quadrupole linear trap (Q3). All the MS, MS/MS, MS^3 and pseudo-MS^3 spectra were obtained in the positive ion mode.

2.4. Fragmentation pattern analysis

The MS/MS and MS^3 data from the unlabeled amines were compared to look for common fragmentation patterns including diagnostic neutral losses and common fragment ions. For the dansyl-labeled compounds, pseudo-MS^3 spectra of the fragment ions at the same m/z value as the protonated original unlabeled amines were generated. Comparison between the pseudo-MS^3 spectra of the labeled amines and the corresponding MS/MS spectra obtained from the unlabeled amines was carried out to determine any similarity in fragmentation patterns before and after dansylation labeling.

3. Results and discussion

The main objective of this work is to determine whether any useful fragment ions can be generated from the dansyl-labeled amines that can be used for structural analysis. Aside from deducing the chemical structure of a completely unknown metabolite, generation of characteristic fragment ions from the dansyl-labeled amines can also facilitate metabolite identification via spectral matching where the fragment ion spectrum of an unknown is searched against those of standards in a library. MS/MS spectral libraries of metabolites and other small molecules have become available in publicly accessible websites [6,8,9]. The number of entries is expected to grow as more compounds are being identified. However, these libraries are generally constructed using unlabeled compounds. To utilize these resources for metabolite identification, it is important that similar types of fragment ions can be obtained from the labeled and unlabeled metabolites. In this work, we first examine the fragmentation pathways or patterns of amine-containing compounds in ESI-MS/MS. We then compare the fragmentation patterns of the unlabeled and labeled amines to determine whether dansylation affects the types and numbers of the fragment ions generated in tandem MS.

For unknown metabolite identification, it is useful to generate as many different fragment ions as possible to produce structural information on different moieties of a molecule. Thus, whenever possible, MS^n of a molecular ion is often conducted. As the QTrap MS instrument has the ability to carry out the MS/MS and MS^3 experiments [10], these fragment ion spectra were generated for the standard amine-containing compounds both before and after dansylation labeling. A total of 32 amine-containing compounds were chosen from the Human Metabolome Database (HMDB) [6], representing a wide range of chemical diversity, with no particular reason or purpose other than the availability of these standards. While these standards only represent a small set of amine-containing metabolites, the observed fragmentation behaviors described in this work should be representative of most amines, with a caution that some exceptions may likely be encountered for some amines. The fragment ion spectra of the 32 labeled and unlabeled compounds are provided in the supplemental material (Supplemental Scheme S1).

3.1. Neutral loss of unlabeled amines

Molecules containing an amino group or primary amines are protonated in positive ion ESI at the nitrogen atom [11] and get

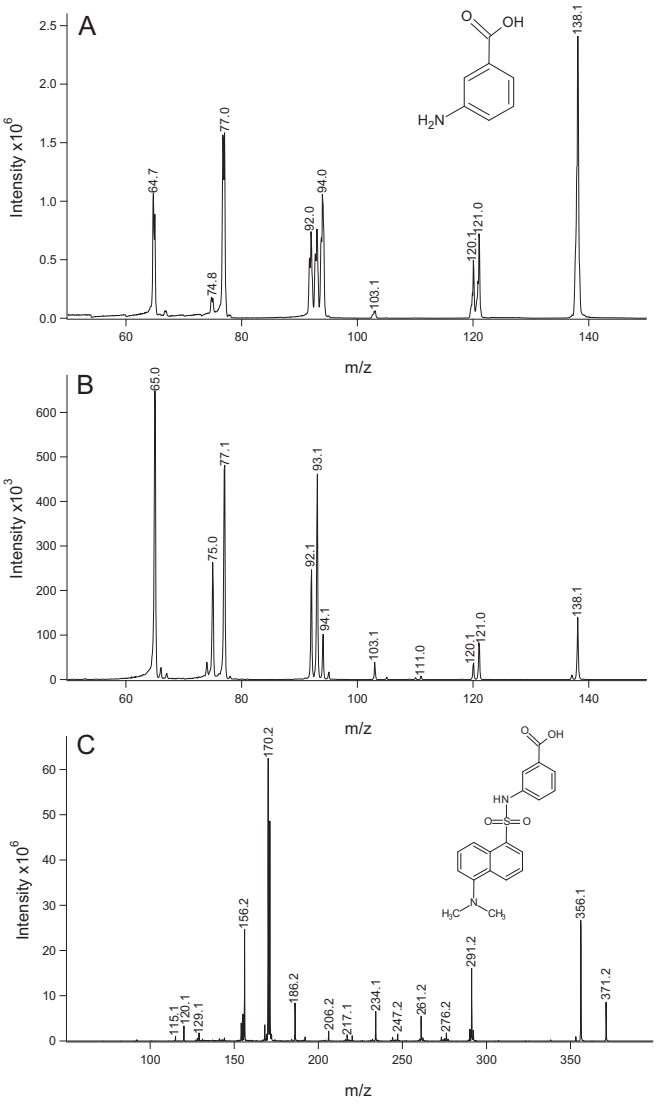

Fig. 1. (A) CID MS/MS spectrum of the protonated 3-aminobenzoic acid. (B) Pseudo-MS^3 spectrum of the skimmer-fragment ion with m/z 138.1 with enhanced product ion (EPI) scan in the QTrap. (C) CID MS/MS spectrum of the protonated dansyl-3-aminobenzoic acid. The collision energy used for each spectrum reported in this work is given in the original spectra presented in Supplemental Scheme S1.

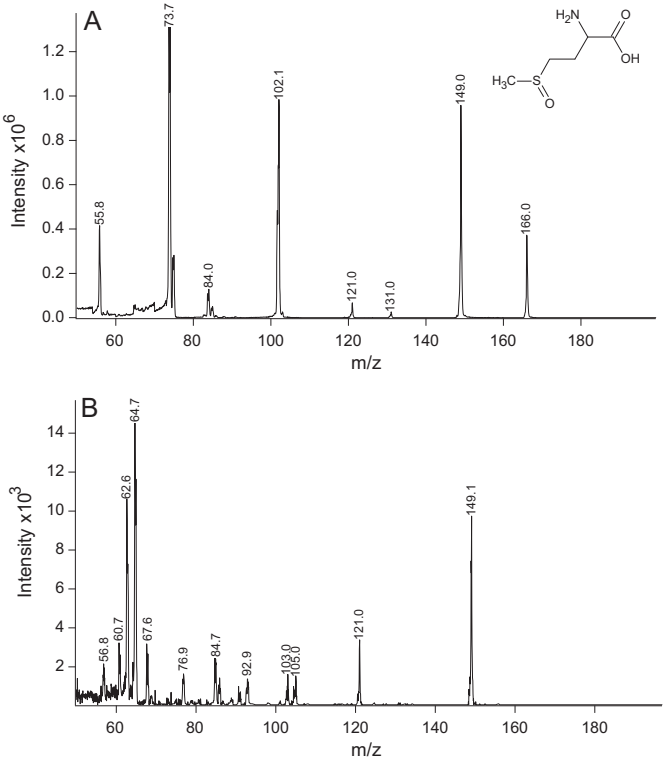

Fig. 2. (A) MS/MS spectrum of the protonated methionine sulfoxide. Peak with m/z 166.0 represents methionine sulfoxide molecular ion after protonation, and peak with m/z 149.0 shows the fragment ion after neutral loss of 17 from the protonated methionine sulfoxide ion. (B) MS^3 spectrum of the deaminated ion of methionine sulfoxide. Peak with m/z 149.1 represents the deaminated ion after neutral loss of 17 from the protonated methionine sulfoxide precursor ion, and peak with m/z 121.0 shows the fragment ion after neutral loss of 28 from the deaminated ion.

cleaved at the C—N bond in CID. As a result, a neutral loss of a nominal mass of 17 Da in the form of NH_3 is observed. Thus, in the MS/MS spectra of primary amines, the molecular ion, $[M+H]^+$, for a singly charged species along with a fragment ion, $[M+H-17]^+$, are commonly observed. One example is shown in Fig. 1A for the CID MS/MS spectrum of 3-aminobenzoic acid (HMDB01891). The peak at m/z 138.1 is from the protonated precursor ion, while the fragment ion at m/z 121.0 is the deaminated ion. Most of the primary amines studied gave neutral loss of 17. However, in the case of serine (see Supplemental Scheme 1, under HMDB00187), the loss of 18 was detected, instead of 17. In this case, intramolecular hydrogen-bond involving the primary amino group can be formed to prevent the loss of amino group in the form of NH_3 [12,13]. The loss of H_2O is a preferred route.

Neutral loss of 17, followed by neutral loss of 28, is also observed both in MS/MS of the protonated amines or MS^3 of the fragment ions from neutral loss of 17 of the protonated amines. This pattern applies mainly to primary amines which also have a carboxy group attached to the same carbon atom, to which the amino group is attached, namely, alpha-amino acid structure. As a primary amine, the charge is retained on the nitrogen atom and cleavage occurs to lose NH_3, resulting in a neutral loss of 17. The resulting deaminated ion, if isolated and fragmented in the MS^3 experiment, can further lose the carbonyl group in the original molecule, giving arise a neutral loss of 28 in the MS^3 spectrum. Panels A and B in Fig. 2 show the MS/MS and MS^3 spectra of methionine sulfoxide (HMDB02005), respectively. The peak at m/z 166.0 is from the protonated parent ion, and the one at m/z 149.0 is from the fragment ion after a neutral loss of 17. Furthermore, the deaminated ion with m/z 149.0 was isolated for further dissociation. In Fig. 2B, the peak at m/z 121.0 is from the fragment ion generated from a neutral loss of 28 from m/z 149.1. In addition, some other neutral losses are also observed in the MS^3 spectrum, such as neutral loss of 44 and 46, corresponding to the loss of CO_2 and HCOOH, respectively. Similar results were obtained for selenomethionine (HMDB03966).

3.2. Fragment ions of unlabeled amines

The MS/MS spectra shown in Figs. 1A and 2A are typical of the CID spectra that can be obtained from the protonated amines. Notably there are several fragment ion peaks detected from low to high masses. These fragment ions can be readily assigned to the chemical structures of the amines. More importantly, these ions provide the chemical signature that is needed for compound identification based on fragment ion spectral match. There are no characteristic core fragment ions, representative of diverse structures of amines, found from the MS/MS spectra of the 32 compounds. However, a couple of interesting observations are worth noting. One is related to a common fragment ion of m/z 72 observed in the MS/MS spectra of long chain

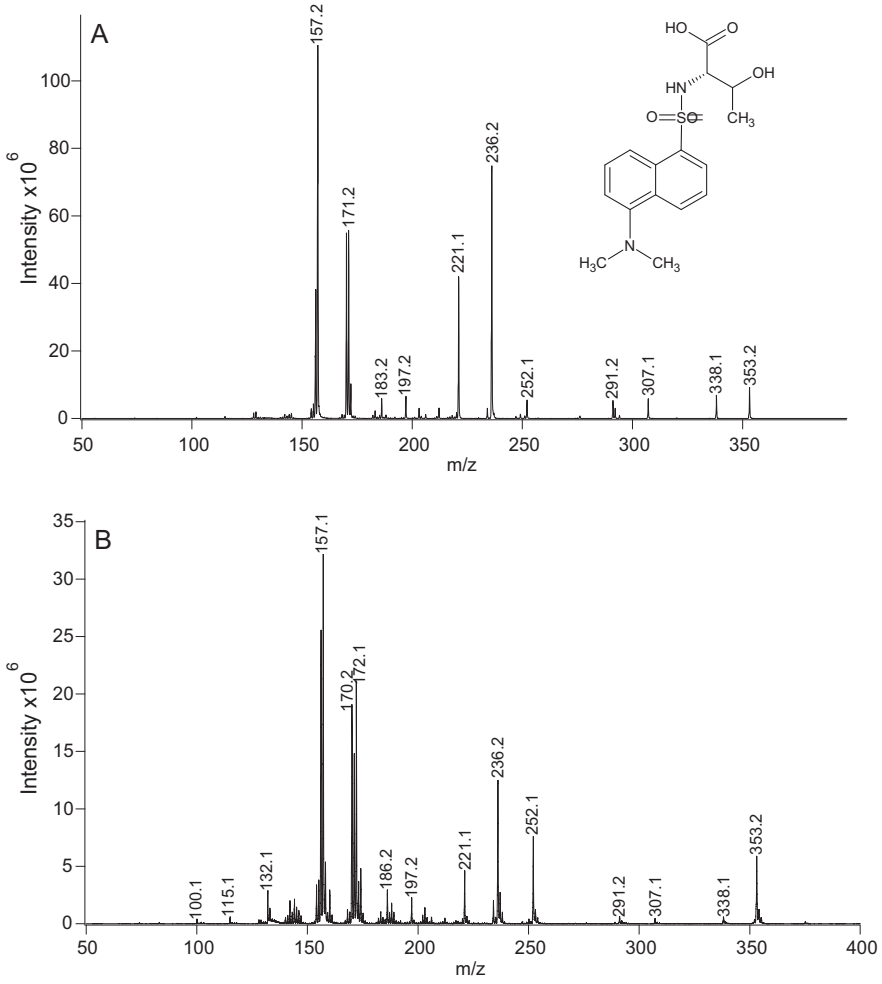

Fig. 3. (A) MS/MS spectrum of the protonated dansyl-threonine. (B) Skimmer-fragmentation spectrum of the protonated dansyl-threonine.

primary amines, such as N-acetylputrescine (HMDB02064) and agmatine (HMDB01432), which has the amino group present at one end of the molecular chain, and connected with four methylene groups ($H_2N{-}CH_2{-}CH_2{-}CH_2{-}CH_2{-}R$). In this type of amine-containing compounds, positive charge is initially retained at the nitrogen atom. Upon CID, cleavage occurs at the C—R bond, forming the ion with the structure of $H_2C{=}CH{-}CH_2{-}CH_2{-}NH_3^+$ at *m*/*z* 72.

Another common fragment ion with *m*/*z* 102 is observed in the MS/MS spectra of primary amines which have the alpha-amino acid structure present in the original molecule and have at least two methylene groups ($-CH_2-CH_2-R$) connected to the carbon atom, to which both the amino group and the carboxy group are attached. As in the case of molecules that give a fragment ion at *m*/*z* 72, in this group of amines, protonation also occurs at the nitrogen atom. Cleavage of the protonated amines occurs at the C—R bond, resulting in the fragment ion which also forms a double-bond between the two methylene groups mentioned above, with its *m*/*z* value of 102. This is exemplified by the MS/MS fragmentation patterns of methionine sulfoxide (HMDB02005), selenomethionine (HMDB03966), and biocytin (HMDB03134).

3.3. MS^2 fragmentation patterns of dansylated amines

For the dansyl-labeled amines, the fragment ions detected in the MS/MS spectra are mostly from the dansyl part, as the charge after protonation is carried by the nitrogen atom in the dansyl moiety. In most cases, there is no fragment ion containing the part belonging to the original amine molecule without the dansyl moiety. In some cases, fragment ions containing the dansyl group from the neutral loss of a moiety from the original amine molecule are observed, which can provide some structural information on the amine molecule.

As an example, Fig. 3A shows the MS/MS spectrum of dansyl-threonine (HMDB00167). The major peaks detected in Fig. 3A can be assigned and the fragmentation scheme of dansyl-threonine is given in Supplemental Scheme 2. Most peaks are from the fragment ions containing the dansyl group. Since the charge is located mainly on the dansyl group, no fragment ions corresponding to the original threonine part are observed in the MS/MS spectrum. In this case, the peak at *m*/*z* 353.2 is from the protonated dansyl-threonine. At the high *m*/*z* region, several fragment ions are detected including the peaks at *m*/*z* 338.1, 307.1 and 291.2. The peak at *m*/*z* 338.1 is from the loss of the CH_3 group in the dansyl moiety. The peak at *m*/*z* 307.1 is generated after losing HCOOH from the original threonine molecule. From the comparison between the MS/MS spectra of the labeled and unlabeled amines, it was also found that the neutral loss fragmentation pathways from the original amine compounds, as described in Section 3.1, can be applied to those of the dansyl-labeled amines. However, since only neutral loss of simple molecules, such as H_2O and HCOOH, from the amine itself are detected in the MS/MS spectra of dansyl-labeled amines, these spectra alone are not sufficient for compound identification with high confidence.

In the MS/MS spectrum of dansyl-threonine, there is no peak detected at the same m/z value as the protonated unlabeled threonine (m/z 119.06). We attempted to use a more energetic skimmer-fragmentation by applying a high declustering potential in the QTrap instrument to dissociate the protonated dansyl-threonine, followed by the detection of the fragment ions generated. Fig. 3B shows the skimmer-fragmentation spectrum. The fragmentation pattern appears to be similar to the CID spectrum shown in Fig. 3A. Although more intense low-mass fragment ions are observed, no peaks corresponding to the protonated threonine or its characteristic fragment ions are detected. Similar results are obtained for the other dansyl-labeled amines and another example is given in Supplemental Scheme 3 for dansyl-tryptophan.

3.4. MS^3 fragmentation patterns of the fragment ions from dansylated amines

The neutral loss fragment ions from the dansyl-labeled amines can be selected for further fragmentation in the linear trap. For example, in Fig. 3A, the ions at m/z 338.1, 307.1 and 291.2 generated by the loss of CH_3, HCOOH, and HCOOH + CH_4, respectively, from the protonated dansyl-threonine, were individually selected for fragmentation. However, the MS^3 spectra of these species (not shown) do not show any further fragment ions from the original amines. Other high-intensity low-mass ions shown in Fig. 3A are mainly from the fragmentation of the dansyl group itself and their MS^3 spectra do not give useful fragment ion information on the original amine molecules.

3.5. Pseudo-MS^3 fragmentation patterns of dansylated amines and comparison to those of unlabeled amines

As it was pointed out above, the MS/MS spectra and the skimmer-fragmentation spectra of dansylated amines do not show fragment ions from the original amine molecules. However, for structural analysis or comparison with the fragment ion spectral library of unlabeled amines, it is critical to produce fragment ions directly from the amines. We resorted to the use of pseudo-MS^3 to produce these fragment ions. Specifically, EPI scans of the second generation of CID product ions of the fragment ions produced from the skimmer-fragmentation of the protonated dansyl amine were conducted to generate the pseudo-MS^3 spectrum for each skimmer-fragment ion. We found that all the neutral loss skimmer-fragment ions containing the dansyl group did not show any characteristic product ions belonging to the original amine molecule, which is consistent with the MS^3 experiment results discussed in Section 3.3. However, by selecting the skimmer-fragment ions with the same m/z value as the unlabeled protonated amine for CID, it was possible to generate a pseudo-MS^3 spectrum containing peaks corresponding to the characteristic product ions that were found in the CID spectrum of the protonated unlabeled amine. While the skimmer-fragmentation spectrum did not show much signal at m/z of the protonated unlabeled amine, it was observed that, by raising the declustering potential to induce skimmer-fragmentation while selecting this particular ion by the first quadrupole mass analyzer, this skimmer-fragment ion could be detected in EPI. This indicates that skimmer-fragmentation did produce this ion, but its intensity was too low to be detected in the skimmer-fragmentation spectrum. However, by selecting this ion for detection or CID, the linear trap allowed sensitive detection of this ion and its product ions. We speculate that the enhanced formation of the protonated unlabeled amine in the skimmer region was likely due to the collision of the dansylated metabolite ions with many types of gaseous species in the skimmer interface, such as solvents, salts and other neutral or ionic molecules.

The above observations are not surprising as, in QTrap, product ion spectra of low-intensity precursor ions can often be generated using selected reaction monitoring (SRM) to trigger EPI scans, even if the precursor ions are not detectable or buried with the background ions using the normal MS scan [10,14]. We note that, in a few cases, such as dansyl-adenine, dansyl-5-hydroxy-L-tryptophan and dansyl-cytosine, the CID spectra as well as the skimmer-fragmentation spectra of the dansyl amines do contain a low-intensity peak with m/z corresponding to the protonated unlabeled amine. For the 32 compounds tested in this work, pseudo-MS^3 spectra of this type of ion can all be generated (see the middle spectrum in each file in Supplemental Scheme 1). One example of such a spectrum is shown in Fig. 1B. Note that, as in Fig. 1, each file in Supplemental Scheme 1 for a particular amine contains three spectra. The spectrum at the top (e.g., Fig. 1A) is the MS/MS spectrum of the protonated unlabeled amine, the one in the middle (e.g., Fig. 1B) is the pseudo-MS^3 spectrum of the fragment ion of the labeled amine which has the same m/z value as the protonated unlabeled amine, and the one at the bottom (e.g., Fig. 1C) is the MS/MS spectrum of the protonated dansyl-labeled amine. By comparing the first and second fragmentation spectra corresponding to the same standard amine, it was found that the fragmentation patterns, in terms of the number and types of fragment ions, before and after dansylation labeling are similar, while for some of the compounds, additional fragment ions can be detected to provide further structural information on the amines.

One example is shown in Fig. 1 for 3-aminobenzoic acid. The MS/MS spectrum (Fig. 1C) of dansyl-3-aminobenzoic acid does not show any characteristic peaks from the fragmentation of the 3-aminobenzoic acid molecule. Based on this MS/MS spectrum alone, it is difficult to confirm the chemical structure of 3-aminobenzoic acid. However, the pseudo-MS^3 spectrum (Fig. 1B) of the ion at m/z 138.1 displays the same types of fragment ions as those observed in the MS/MS spectrum of the unlabeled 3-aminobenzoic acid at m/z 138.1 (Fig. 1A). Thus, in this case, the pseudo-MS^3 spectrum would provide the needed fragment ion information to identity this compound, if this were an unknown metabolite.

Another example is shown in Fig. 4 for N-acetylputrescine (HMDB02064). Fig. 4A shows the MS/MS spectrum of the protonated N-acetylputrescine, in which the peak at m/z 131.2 is from the protonated N-acetylputrescine precursor ion, while the peaks at m/z 114.0 and 71.8 represent the two fragment ions generated. Fig. 4B shows the pseudo-MS^3 spectrum of the skimmer-fragment ion generated from the dansyl-N-acetylputrescine ion with the same m/z value as that of the protonated N-acetylputrescine. In this spectrum, the fragment ions with m/z 114.1 and 72.1 are the same ions generated as above, while the peak at m/z 103.1 is from an extra fragment ion generated from the labeled compound (the fragmentation scheme for peak assignment is shown in Supplemental Scheme 4). Note that, in this particular case, the same fragment ions at m/z 114.1 and 72.1 are detected in the skimmer-fragmentation spectrum as shown in Fig. 4C. However, the pseudo-MS^3 spectrum generated from the precursor ion at m/z 131.2 is more reliable in determining or confirming the chemical structure of N-acetylputrescine, particularly when the MS/MS spectrum of the protonated N-acetylputrescine is known.

Among the 32 compounds investigated, all, except one, give similar fragmentation patterns in their pseudo-MS^3 spectra to those from the MS/MS spectra of unlabeled amines. The exception is for dansyl-agmatine (HMDB01432). In this case, the characteristic skimmer-fragment ions with m/z corresponding to the protonated agmatine was detected (see the middle spectrum in Supplemental Scheme 1, under HMDB01432). However, little fragment ions were detected. Fortunately, for this compound, the MS/MS spectrum of dansyl-agmatine itself contains the two major fragment ion (m/z 72.1 and 114.1) (see the bottom spectrum in Supplemental Scheme

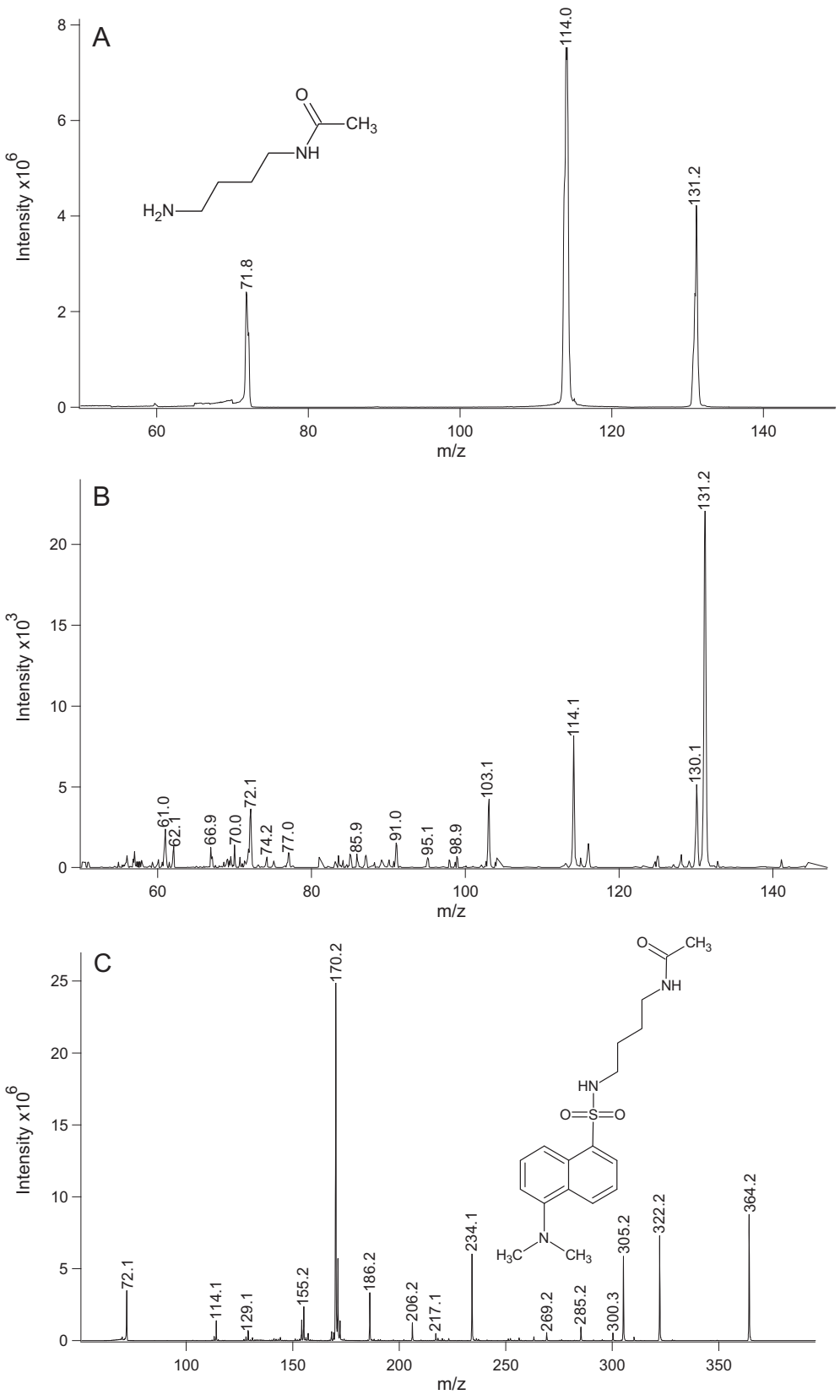

Fig. 4. (A) MS/MS spectrum of the protonated N-acetylputrescine. (B) Pseudo-MS^3 spectrum of the skimmer-fragment ion with *m*/*z* 131.2 with enhanced product ion (EPI) scan in QTrap. (C) MS/MS spectrum of the protonated dansyl-N-acetylputrescine.

1) that can be used for identification of this compound. The reason of this compound did not give a good pseudo-MS^3 spectrum is unknown. It may be related to the structure of this unique molecule where the amine group may form a stable structure through a hydrogen bridge with another nitrogen in the guanidine moiety. During the dansyl-agmatine dissociation process, this stable structure might be formed while in the protonated form as in MS/MS of the unlabeled amine a more open structure was formed.

3.6. Relevance to metabolome profiling

Skimmer fragmentation is not widely used for structural analysis due to the lack of precursor ion selection. However, for this work, we use the skimmer fragmentation to generate the precursor ion of the unlabeled metabolite from the dansylated metabolite, followed by selecting the precursor ion using the first mass analyzer for further fragmentation to generate the MS/MS spectrum. Thus, the precursor selection of the more useful, unlabeled metabolite ion is still performed. As a result, we generate the MS/MS spectrum of the precursor ion of the unlabeled metabolite, which is more important than the precursor ion of the labeled metabolite.

Our results indicate that a pseudo-MS^3 spectrum of the skimmer-fragment ions that has the same m/z value as the protonated unlabeled amine can be used for structural analysis. The intensity of the protonated unlabeled amine generated is generally low. As a result, the sensitivity of the pseudo-MS^3 method is not as good as the MS detection of the intact dansylated amine or the MS/MS spectral acquisition from the dansylated amine molecular ion. However, this low sensitivity in producing the pseudo-MS^3 spectrum should not be a major barrier in the overall workflow of the dansylation LC–MS metabolome profiling method. In the dansylation LC–MS method, quantitative metabolome profiling of many comparative samples (e.g., diseased vs. healthy group) is carried out first based on the MS data only, not the MS/MS data. Statistics analysis of the relative metabolome abundance differences among these samples is then conducted to determine which metabolite features (each feature with specific retention time and accurate mass) give the most separation of the two groups. Usually only a dozen or so of these features are found. At last, research efforts will be devoted to the identification of these features, i.e., determining the structures of these metabolites. Thus, the proposed pseudo-MS^3 approach is only used in the last step. To compensate for the low sensitivity of the method, samples can be pooled to increase the analyte concentration for LC–MS injection. Alternatively, the LC fraction containing the important metabolite features is collected from a larger column separation or from multiple injections of a small column separation, followed by pseudo-MS^3 analysis of the collected fraction.

It should also be noted that there are some significant advantages of using the pseudo-MS^3 spectra for structural analysis over the use of MS/MS spectra of dansylated compounds. As it was shown earlier, the MS/MS spectra of many dansylated metabolites were not informative about the original structures of the amines (e.g., only loss of H_2O or CO_2 was found). Thus, the usefulness of these spectra for spectral match with the MS/MS spectra of dansylated amine standards for unknown metabolite identification is questionable. In contract, the pseudo-MS^3 spectra contain the peaks of fragment ions with the structural signatures of the original amines and, thus, spectral match between the pseudo-MS^3 spectrum and the MS/MS spectrum of an amine standard is more reliable for compound identification. In addition, the pseudo-MS^3 spectra can be, in principal, used to facilitate unknown metabolite identification, even if the MS/MS spectrum of the unknown is not present in the spectral library of unlabeled standards. In this case, comparison of fragmentation patterns of different compounds with similar structures (e.g., a core structure with CH_3O— attached in the standard library vs. the same core structure with CH_3CH_2O— attached in the unknown sample) can be used to reduce the number of metabolite candidates of an unknown metabolite.

4. Conclusions

We have investigated the fragmentation behaviors of 32 dansyl-labeled amines with diverse chemical structures and their unlabeled counterparts using an ESI QTrap mass spectrometer. It was found that the MS/MS spectra of dansyl-labeled amines mainly consist of peaks from the fragment ions containing the dansyl group. In most cases, there were a few fragment ions observed from the loss of H_2O, NH_3, HCOOH from the original amine molecule. These ions are usually not informative for deducing or confirming the chemical structures of amines. In a few cases, fragment ions from the original amine molecule were detected. However, they are not sufficient to identify a compound, if spectral match to a spectral library composed of fragment ion spectra of unlabeled amines is used. In contrast, a pseudo-MS^3 spectrum of the skimmer-fragment ion that has the same m/z value as the protonated unlabeled amine could be generated for structural analysis. In most cases, these spectra contain the same types of fragment ions as those in the MS/MS spectra of the protonated unlabeled amines, while in a few cases additional types of fragment ions are observed. We suggest that the pseudo-MS^3 spectra generated from the dansyl-labeled amines could be used for metabolite identification in a workflow where the metabolome is labeled with dansylation chemistry for comprehensive profiling. The pseudo-MS^3 spectra can be directly compared to the library spectra of unlabeled amines for potential spectral matching. The application of this approach for metabolome analysis will be reported in the future.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ijms.2012.02.019.

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