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Determination of Double Bond Location in Fatty Acids by Manganese Adduction and Electron Induced Dissociation

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Double bond locations in fatty acids can be determined from characteristic charge-remote fragmentation patterns of alkali metal-adducted fatty acids following high energy collision activated dissociation (CAD). With low energy CAD, several chemical derivatization methods, including ozonization, epoxidation, and hydroxylation, have been used to generate characteristic fragments. However, high energy CAD is not universally available and involves a high degree of scattering, causing product ion loss. Further, derivatization reactions involve side reactions and sample loss. Here, we analyzed metal-adducted fatty acids to investigate the utility of electron induced dissociation (EID) for determining double bond location. EID has been proposed to involve both electronic excitation, similar to high energy CAD, and vibrational exciation. Various metals (Li, Zn, Co, Ni, Mg, Ca, Fe, and Mn) were investigated to fix one charge at the carboxylate end of fatty acids to promote charge-remote fragmentation. EID of Mn(II)-adducted fatty acids allowed determination of all double bond locations of arachidonic acid, linolenic acid, oleic acid, and stearic acid. For Mn(II)-adducted fatty acids, reduced characteristic charge-remote product ion abundances at the double bond positions are indicative of double bond locations. However, other metal adducts did not generally provide characteristic product ion abundances at all double bond locations.

Polyunsaturated fatty acids are essential for cell membrane functioning because many membrane properties, such as fluidity and permeability, are closely related to the level of unsaturation.[1,2] Lipid peroxidation results in loss of membrane polyunsaturated fatty acids.[1] Also, when an oil or fat becomes oxidized, health concern is due to the potential production of free radicals, which can be highly carcinogenic.[3−6] Double bond sites in unsaturated fatty acids and lipids are plausibly oxidized and form free radicals, which can cause tissue damage and alterations in cell membranes.[1,3,4,7,8] Thus, the identification of double bond locations in fatty acids can be beneficial for understanding lipid biology and also its related disease states.[1,2]

Double bond locations in aliphatic compounds, including fatty acids, can be obtained from mass spectrometry (MS).[9−14] Such information can be determined by charge-remote fragmentation processes of alkali metal-adducted fatty acids in high energy collisional activation with fast atom bombardment (FAB) ionization.[9,15] However, FAB desorption of fatty acid mixtures can result in preferential desorption of some ions, chemical noise, and low sensitivity.[17,19] In addition, sector-type instruments for high energy collision activated dissociation (CAD) are not universally available, and high energy CAD involves a high degree of scattering, causing product ion loss.[20] Recently, low energy CAD of Cu(II)-adducted fatty acids in an ion trap instrument was used to provide diagnostic product ions to aid the identification of double bond locations in unsaturated fatty acids;[32] however, double bond localization remains challenging for monounsaturated fatty acids.

Charge remote fragmentation occurs remote from a charge site and appears to readily occur in high energy CAD of fatty acids, lipids, steroids, and other compounds containing long alkyl chains.[15,17,33] Charge remote fragmentation has been used to provide double bond positions of fatty acids from FAB-MS/MS (high energy CAD) of lithiated fatty acids using sector-type mass spectrometry by Gross and others.[17,33,34] More recently, tandem time-of-flight (TOF/TOF) MS was applied to determine double bond locations of lithiated fatty acids by McEwen and co-workers using solvent-free matrix-assisted laser desorption ionization (MALDI).[10] Charge-remote bond cleavages in fatty acids are

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reduced in the vicinity of double bonds, thus providing information on double bond locations.[16] Fixed charge sites are important for predominant charge-remote fragmentation processes because charge-driven processes compete with charge-remote processes. In charge-driven fragmentation, charge migration results in rearrangement of chemical structure, making identification of double bond positions impossible.[15] High energy CAD is known to involve electronic excitation as a dominant process for generating charge-remote product ions, and vibrational/rotational excitation is considerably less efficient.[16]

Electron induced dissociation (EID) involves interactions between singly charged analyte ions and free electrons. This concept was first shown with 3−9 eV electrons in 1979 by Cody and Freiser for radical cations.[21] EID does not require multiply charged precursor ions, thereby differing from other ion−electron interactions such as electron capture dissociation (ECD)[22−25] and electron detachment dissociation (EDD).[26−28] Consequently, EID is compatible with smaller biomolecules (such as fatty acids) for which formation of gas-phase multiply charged ions is energetically unfavorable. Zubarev and co-workers applied EID (10−13 eV electron irradiation) to singly charged oligosaccharide cations,[29] and we have applied EID for structural characterization of phosphate-containing metabolites.[30] EID of phosphate-containing metabolites provided complementary structural information compared to CAD and infrared multiphoton dissociation (IRMPD) and generally generated more extensive fragmentation than the latter two techniques.[44] O'Hair and co-workers have proposed that EID occurs via electronic and vibrational excitation, based on similarities between the types of product ions observed from EID, ultraviolet photodissociation, and electron ionization (EI) mass spectra.[31] Thus, EID may be an alternative technique to high-energy CAD for revealing information on double bond locations in fatty acids. To our knowledge, EID has not previously been applied toward fatty acid analysis. We used several metals (Li, Zn, Co, Ni, Mg, Ca, Fe, and Mn) to fix a positive charge at the end of fatty acids. Mn(II) adduction consistently generated charge-remote fragmentation in EID. The charge-remote product ion abundances at each carbon location were compared to deduce double bond positions. EID of Mn(II)-adducted arachidonic acid was compared to IRMPD of the same species to illustrate that EID involves both electronic and vibrational excitation in contrast to IRMPD which only involves the latter.

EXPERIMENTAL SECTION

Sample Preparation. Fatty acids used in this work include stearic acid, oleic acid, linolenic acid, and arachidonic acid. Fatty acids and metal salts, including $MnCl_2$, $CoBr_2$, and $NiBr_2$, were purchased from Sigma-Aldrich (St. Louis, MO). Fatty acid (70−200 μM) was mixed with 200−600 μM metal salt in methanol/water (80/20 v/v). Sample solutions of metal (Met)-adducted fatty acids were freshly made 10−30 min prior to MS analysis.

Fourier Transform Ion Cyclotron Resonance Mass Spectrometry. Singly charged metal-adducted fatty acids, $[M + Met - H]^+$ ($[M + 2Met - H]^+$ for Li), were generated by external electrospray ionization (ESI) at 70 μL/h (Apollo II dual stage ion funnel ion source, Bruker Daltonics, Billerica, MA). All experiments were performed with a 7 T quadrupole (Q)-FTICR mass spectrometer (APEX-Q, Bruker Daltonics) as previously described.[28] All data were obtained in positive ion mode. Briefly, ions produced by ESI were mass-selectively externally accumulated[32,33] in a hexapole for 0.1−2 s, transferred via high voltage ion optics, and captured in the ICR cell by dynamic trapping. This accumulation sequence was looped three times to improve precursor ion abundance. In MS/MS experiments, mass selective external accumulation of $[M + Met - H]^+$ ($[M + 2Met - H]^+$ for Li) was employed. In some cases, mass selective external accumulation was followed by further isolation via correlated harmonic excitation fields (CHEF)[34] inside the ICR cell to eliminate unwanted peaks caused by impurities and byproducts from adduct forming reactions. An indirectly heated hollow dispenser cathode was used for electron generation.[35] A heating current of 1.8 A was applied to a heater element located behind the cathode. For EID, performed inside the ICR cell, the cathode bias voltage was pulsed to 25−50 eV for 50−500 ms. IRMPD was performed inside the ICR cell with a 25 W, 10.6 μm, CO_2 laser (Synrad, Mukilteo, WA). The laser beam was deflected by two mirrors for alignment through the hollow dispenser cathode to the center of the ICR cell. The beam entered the vacuum system through a BaF_2 window. Photon irradiation was performed for 300−600 ms at 8.75−10 W laser power. All mass spectra were acquired with XMASS software (version 6.1, Bruker Daltonics) in broadband mode from m/z 21 to 1000 with 256 K data points and summed over 10−30 scans. Data processing was performed with the MIDAS analysis software.[36] Calculated masses of precursor ions, $[M + Met - H]^+$ ($[M + 2Met - H]^+$ for Li), and one of the most abundant product ions were used for internal calibration.

RESULTS

Charge-Remote Fragmentation in EID of Mn(II)-Adducted Fatty Acids. Figure 1 shows EID spectra of Mn(II)-adducted arachidonic acid, linolenic acid, oleic acid, and stearic acid, where

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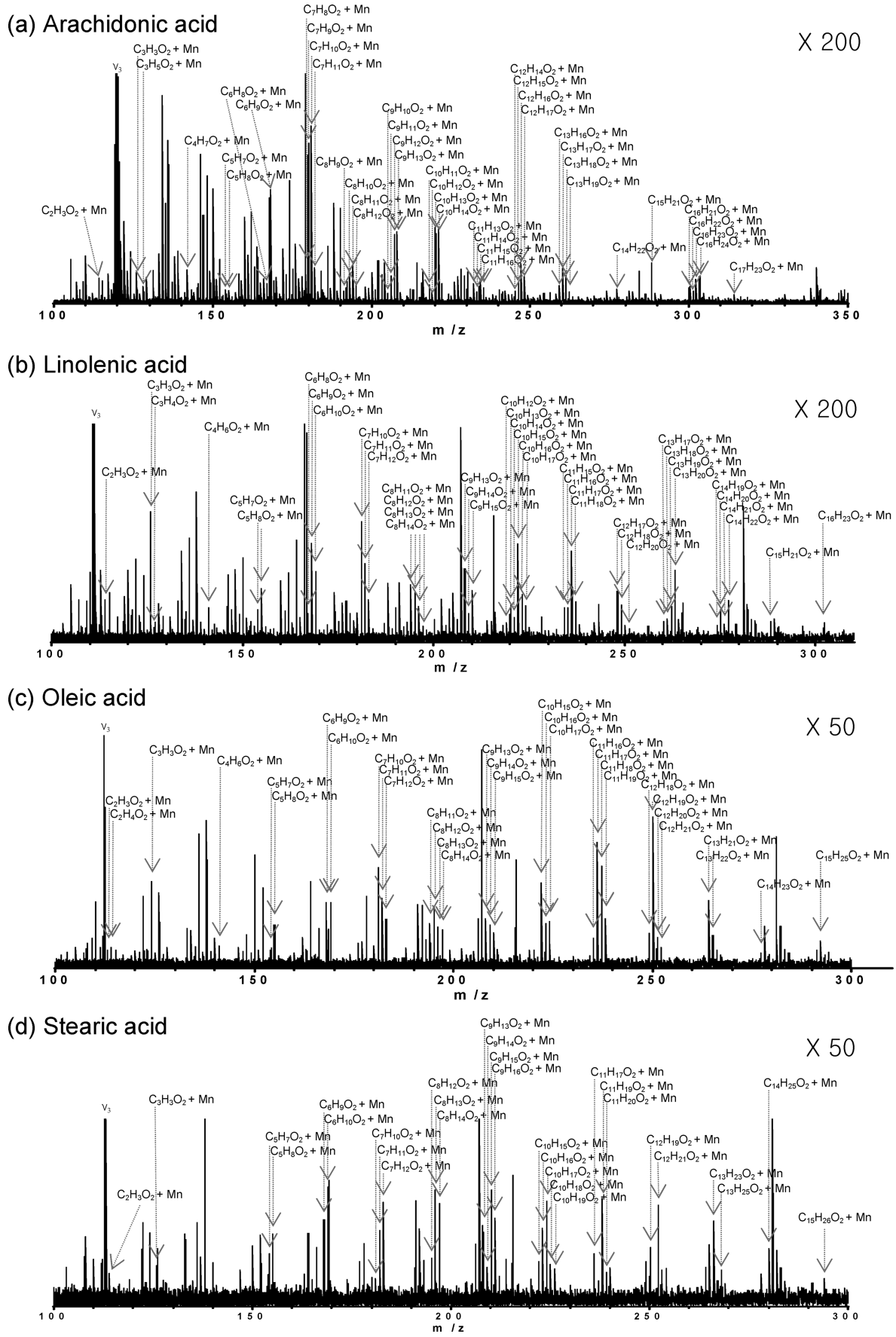

Figure 1. EID of Mn(II)-adducted fatty acids: (a) arachidonic acid ($d = 4$), (b) linolenic acid ($d = 3$), (c) oleic acid ($d = 1$), and (d) stearic acid ($d = 0$), where d indicates the number of double bonds in each fatty acid. Only charge-remote product ion peaks, $[C_xH_yO_2 + Mn]^+$, are labeled. The precursor ion peaks, $[M + Mn - H]^+$, are outside the displayed m/z range. The Y-axis is zoomed 50 or 200 times as indicated by "×50" or "×200".

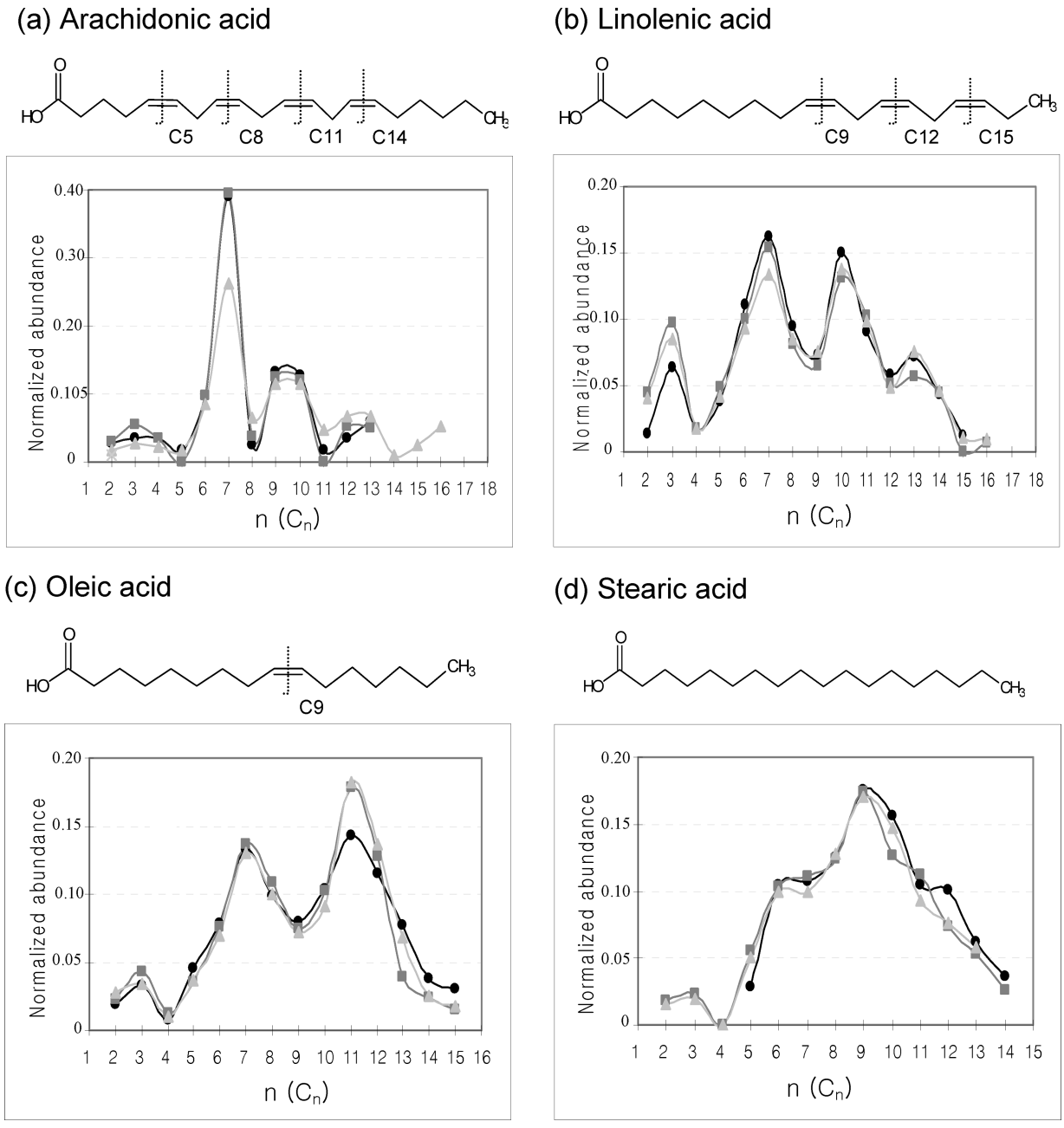

Figure 2. Normalized product ion abundances of $[C_xH_yO_2 + Met]^+$ vs C_n (*n* denotes carbon position from the carboxylate end of a fatty acid) in EID of Mn(II)-adducted fatty acids: (a) arachidonic acid ($d = 4$), (b) linolenic acid ($d = 3$), (c) oleic acid ($d = 1$), and (d) stearic acid ($d = 0$), where *d* indicates the number of double bonds in each fatty acid. Three observations were made for each Mn(II)-adducted fatty acid.

each fatty acid has 4, 3, 1, and 0 double bonds, respectively. In this figure, only characteristic charge-remote fragments, $[C_xH_yO_2 + Mn]^+$, are labeled whereas unlabeled peaks are mostly due to charge-driven fragmentation, including $[C_xH_y + Mn]^+$. Charge-remote product ion abundances at each carbon−carbon cleavage site were calculated by adding all of the $[C_xH_yO_2 + Met]^+$-type ion abundances at each carbon position and normalizing to the total $[C_xH_yO_2 + Met]^+$-type ion abundances in the EID spectrum. In EID of Mn(II)-adducted fatty acids, even and odd electron species were observed, generated by heterolytic (resulting in product ions of, e.g., the types $[C_nH_{2n-1}O_2 + Mn]^+$, $[C_nH_{2n-3}O_2 + Mn]^+$, and $[C_nH_{2n-5}O_2 + Mn]^+$) and homolytic bond cleavages (producing, e.g., the product ion types $[C_nH_{2n-2}O_2 + Mn]^+$, $[C_nH_{2n-4}O_2 + Mn]^+$, and $[C_nH_{2n-6}O_2 + Mn]^+$), respectively. Similar behavior was noted in high energy CAD of ESI-generated precursor ions, while mostly heterolytic bond cleavages were observed in FAB-high energy CAD.[37]

For clarity, Supplementary Table 1 (Supporting Information) shows peak assignments for charge-remote product ions from EID of Mn(II)-adducted ararchidonic acid as well as an example (C_5 position) of how normalized charge-remote product ion abundances were calculated. Double bond locations can be identified from normalized product ion abundances at each carbon site, as shown in Figure 2. EID experiments for each fatty acid were

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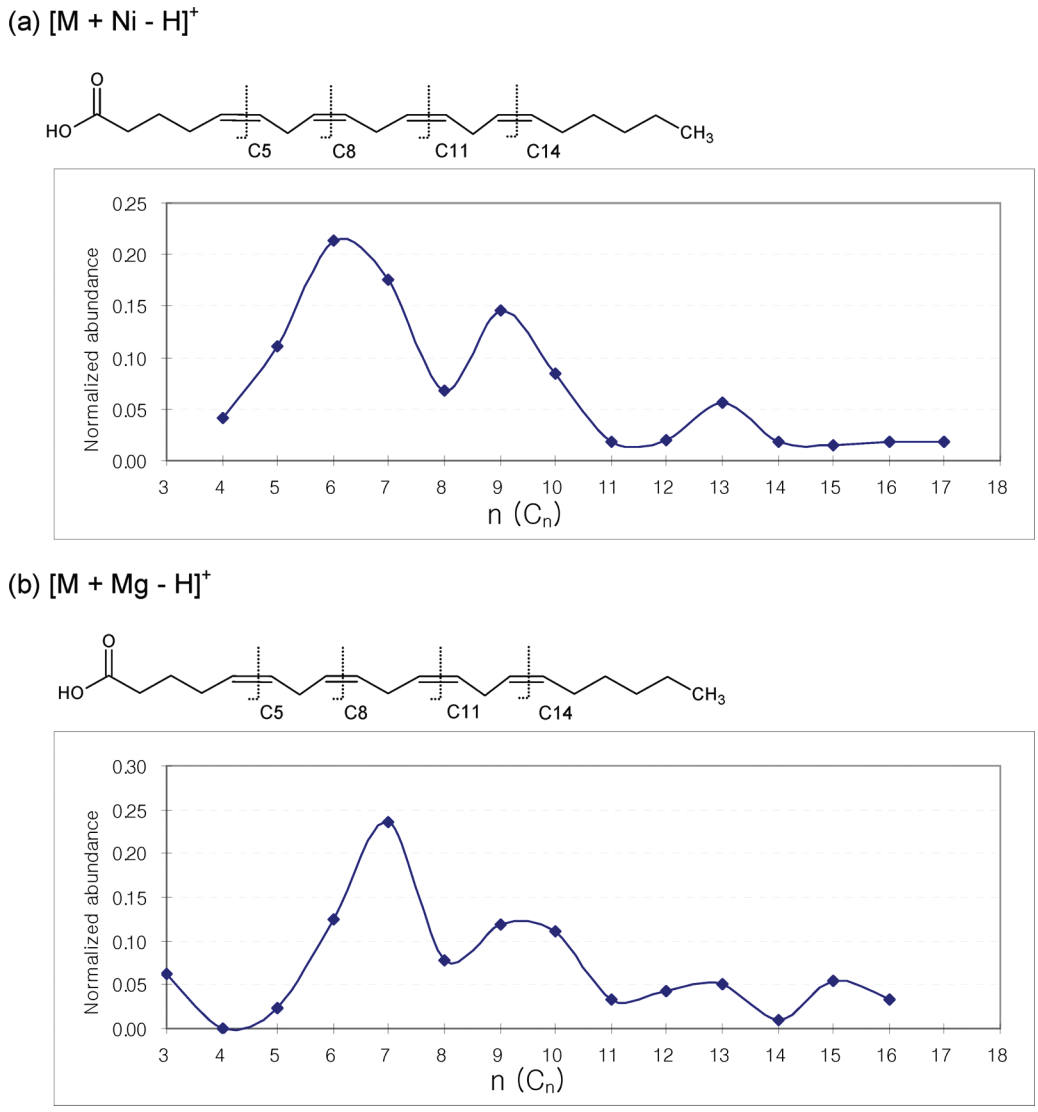

Figure 3. Normalized product ion abundances of $[C_xH_yO_2 + Met]^+$ vs C_n (*n* denotes carbon position from the carboxylate end of a fatty acid) in EID of Ni(II)- and Mg(II)-adducted arachidonic acid. The structure of arachidonic acid is shown with double bond locations.

repeated three times on three different days to verify the reliability of EID as a method for double bond localization in fatty acids. As shown in Figure 2, EID spectra of Mn(II)-adducted fatty acids provided highly reproducible structural information regarding double bond positions for each fatty acid. Lower product ion abundances at the C_4 position, observed in EID of Mn(II)-adducted linolenic acid, oleic acid, and stearic acid, indicated that those fatty acids do not have double bonds between C_5 and C_6. In contrast, the lower product ion abundance at C_5 compared to C_4 was indicative of the existence of a double bond between C_5 and C_6 in arachidonic acid. Other double bond locations in each fatty acid could be obtained from valley positions in the graph of normalized charge-remote product ion abundances vs carbon position (C_n), as shown in Figure 2. Gas-phase ion fragmentation reactions are charge remote when there is no important interaction between the charge and the cleavage sites. However, hydrocarbon chains are flexible, and such interactions can, therefore, occur even in the presence of a terminal fixed charge, particularly at less remote sites.[15] Gross and co-workers have reported that some product ions formed by cleavage near the charge are stabilized by a cyclic conformation.[15] For example, C_4-C_5 bond cleavage in deprotonated palmitic acid was enhanced due to formation of a cyclic structure.[18] Similarly, enhanced cleavage at the C_3 position occurred in EID of Mn(II)-adducted linolenic, oleic, and stearic acid (Figure 2b–d), possibly due to ring formation.

Mn(II) is expected to bind tightly with the carboxylate anion end of fatty acids because both Mn(II) and the carboxylate anion have hard Lewis acid and base properties, respectively. Binding energies of divalent metal ions and H_2O/OH^- increase sharply at the transition between d^0 (Ca(II)) and d^n (Sc(II)) due to electron occupation in d orbitals.[38] These chemical properties may explain why Mn(II) appears to be more efficient than other divalent metals examined (see below) for "fixing" a charge at the carboxylate end of fatty acids.

Charge-Remote Fragmentation in EID of Arachidonic Acid Adducted with Metals Other than Mn(II). In addition to Mn(II), which yielded successful charge remote fragmentation in EID (see above), Li(I) and other divalent metals (Zn(II), Co(II), Ni(II), Mg(II), Ca(II), and Fe(II)) were examined as adducts in EID of

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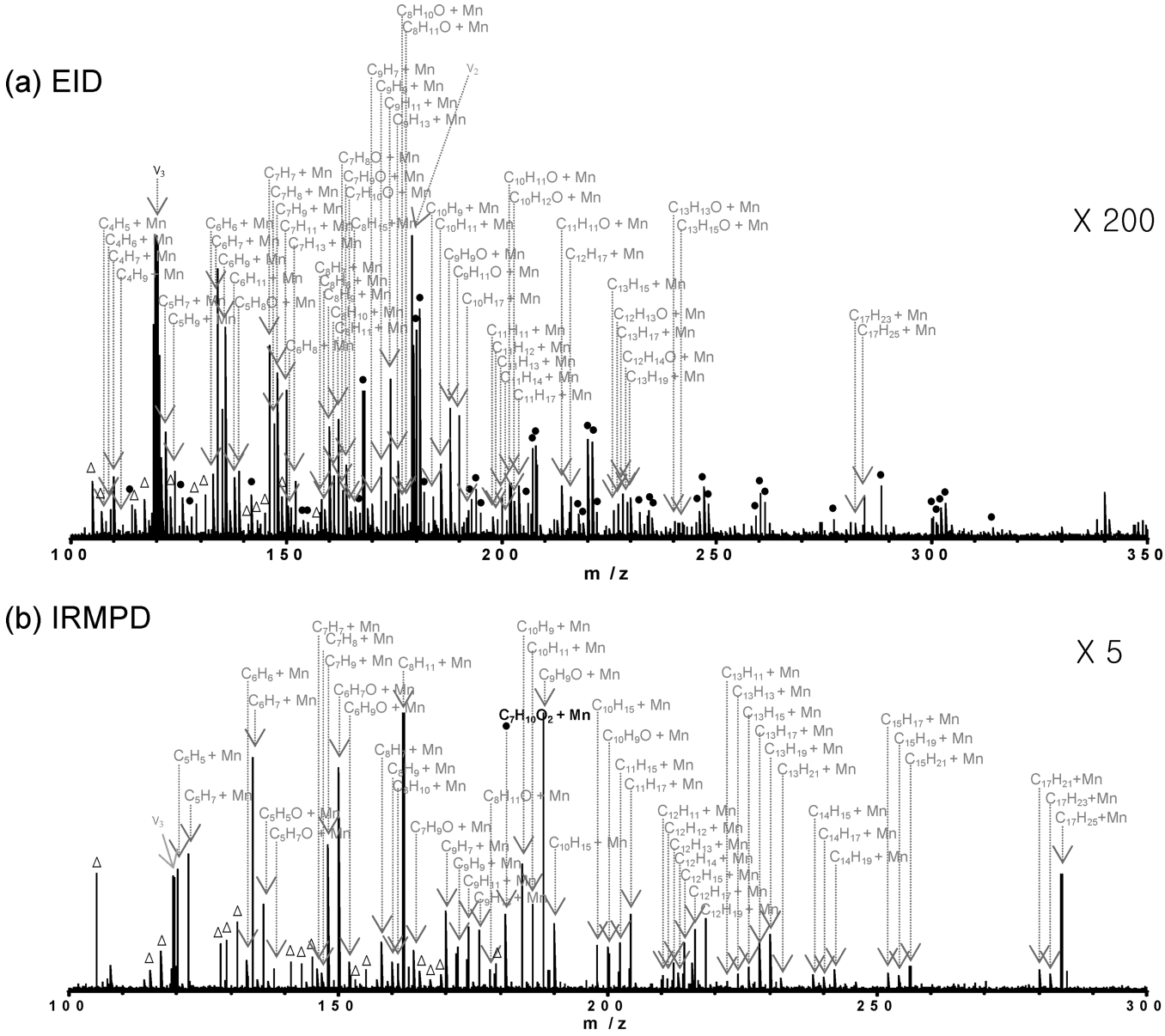

Figure 4. EID (a) and IRMPD (b) of Mn(II)-adducted arachidonic acid. ν_2 and ν_3 denote the second and third harmonic peaks. $[C_xH_yO_2 + Mn]^+$ product ion peaks are labeled with "●", and $[C_xH_y]^+$-type peaks are labeled with "△". Figure 4a shows the same spectrum as in Figure 1a, but here, all peaks are labeled for comparison with the IRMPD spectrum. *Y*-axis is zoomed 200 or 5 times, indicated by "×200" or "×5".

arachidonic acid. Lithium was our first metal of choice because doubly Li(I)-adducted fatty acids, $[M + 2Li - H]^+$, have been shown to yield charge-remote fragmentation in high energy CAD.[9,10,16,18] Electron irradiation (~26 eV electrons) of the $[M + 2Li - H]^+$ form of arachidonic acid did not yield any product ions (Supplementary Figure 1a, Supporting Information). One explanation for this discrepancy may be the different means of electronic excitation in EID vs high energy CAD: the latter involves interactions between ions and neutrals whereas the former involves ion−electron interactions. Thus, physical parameters such as polarizibility (which is low for lithium) should be more important in EID compared to high energy CAD.

EID (~20 eV electrons) of Zn(II)- and Co(II)-adducted arachidonic acid yielded very limited charge-remote fragmentation (Supplementary Figure 1b,c, Supporting Information). In contrast, EID of Ni(II)-, Mg(II)-, Ca(II)-, and Fe(II)-adducted arachidonic acid provided extensive charge-remote product ions of the type $[C_xH_yO_2 + Met]^+$. (As an example, EID of Ni(II)-adducted arachidonic acid is shown in Supplementary Figure 1d, Supporting Information.)

Figure 3 shows charge-remote product ion abundances as a function of alkyl chain carbon position from EID spectra of Ni(II)- and Mg(II)-adducted arachidonic acid. Similar to the Mn(II) data in Figure 2, $[C_xH_yO_2 + Met]^+$-type product ions were used to generate this plot. EID of Ca(II)- and Fe(II)-adducted arachidonic acid provided very similar results; thus, the corresponding data are not shown. EID of these metal-adducted arachidonic acid did not provide characteristic ion abundance patterns at all double bond positions. The double bond (C_{14}−C_{15}) far from the carboxylate end could not be identified from EID of Ni(II)-adducted arachidonic acid (Figure 3a). Further, the double bond (C_5−C_6) close to the carboxylate end could not be determined from EID

of Ni(II)- nor Mg(II)-adducted arachidonic acid (Figure 3a,b). We hypothesize that Ni(II) or Mg(II) adduction may yield a "less fixed" charge compared to Mn(II) adduction, thereby failing to provide all double bond locations in EID.

EID of Mn(II)-adducted arachidonic acid was compared to IRMPD of the same species. In contrast to EID (believed to occur via both electronic and vibrational activation), dissociation in IRMPD occurs solely via vibrational excitation. The mixture of mostly $[C_xH_y + Mn]^+$ and $[C_xH_yO_2 + Mn]^+$ species observed in EID (Figure 4a) implies competition between charge-driven and charge-remote processes. However, IRMPD of the same precursor ions mainly yielded product ions from charge-driven fragmentation, $[C_xH_y + Mn]^+$, as expected from vibrational activation (Figure 4b). In contrast, high energy CAD (known to involve electronic excitation) provides mainly charge-remote product ions.[9] The internal energy required for charge-remote fragmentation is estimated to be ~1.4−2.9 eV for protonated fatty acids.[39] In 70 eV EI, molecular ions of small alkenes were found to be isomerized to a mixture of interconverting structures.[40] We propose that the 25−50 eV electron energies used in EID are sufficient to promote charge-remote fragmentation but not high enough to cause isomerization of Mn(II)-adducted fatty acids, which would result in double bond migration.

CONCLUSION

Mn(II)-adducted fatty acids were analyzed to investigate the utility of EID for determining double bond locations. Charge-remote product ion abundances of $[C_xH_yO_2 + Mn]^+$-type fragments generated by EID are significantly reduced at double bond positions. Analysis of $[C_xH_yO_2 + Mn]^+$-type product ion abundances from EID of Mn(II)-adducted fatty acids allowed determination of all double bond positions. However, other metal adducts did not generally provide characteristic product ion abundances at all double bond locations. The resulting structural information on double bond locations for Mn(II)-adducted fatty acids may be explained by dominant electronic excitation processes in EID and efficient generation of a fixed charge at the carboxylate end due to strong interaction between Mn(II) cation and carboxylate anion. EID of Mn(II)-adducted arachidonic acid was compared with IRMPD of the same species. As expected, mostly charge-driven fragmentation was observed in IRMPD whereas both charge-remote and charge-driven product ions were observed in EID. In contrast, high energy CAD is known to occur mainly via electronic excitation and results in dominant charge-remote product ions.

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SUPPORTING INFORMATION AVAILABLE

Peak assignments and calculations for Figures 1a and 2a. EID spectra of Li-, Zn-, Co-, and Ni-adducted arachidonic acid. This material is available free of charge via the Internet at http://pubs.acs.org.

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