Published in final edited form as:

J Mass Spectrom. 2017 May ; 52(5): 271–282. doi:10.1002/jms.3926.

Charge transfer dissociation (CTD) of phosphocholines: gas-phase ion/ion reactions between helium cations and phospholipid cations

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Abstract

Phospholipid cations formed by electrospray ionization were subjected to excitation and fragmentation by a beam of 6 keV helium cations in a process termed charge transfer dissociation (CTD). The resulting fragmentation pattern in CTD is different from that of conventional collision-induced dissociation (CID), but analogous to that of metastable atom-activated dissociation (MAD) and electron-induced dissociation (EID). Like CID, CTD yields product ions indicative of acyl chain lengths and degrees of unsaturation in the fatty acyl moieties, but also provides additional structural diagnostic information, such as double bond position. Although CTD hasn't been tested on a larger lipid sample pool, the extent of structural information obtained demonstrates that CTD is a useful tool for lipid structure characterization, and a potentially useful tool in future lipidomics workflows. CTD is relatively unique in that it can produce a relatively strong series of 2+ product ions with enhanced abundance at the double bond position. The generally low signal-to-noise ratios and spectral complexity of CTD make it less appealing than OzID or other radical-induced methods for the lipids studies here, but improvements in CTD efficiency could make CTD more appealing in the future.

Introduction

Lipids are essential components of cellular membranes in living cells.^[1, 2] In addition to serving as a “container” for the cell, lipids also show remarkable involvement in a range of lipid-lipid and lipid-protein interactions, thus acting as key players with distinctive biochemical roles and biophysical properties.^[3] A detailed description of all lipids and their functions at the cellular level would greatly facilitate the understanding of signaling, lipid metabolism, and membrane vesicle trafficking. However, the full structural characterization and quantitation of all lipids in a given system remains a formidable challenge to biochemists.^[4]

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Mass spectrometry (MS) has emerged as an indispensable analytical tool for the structural characterization of lipids. Soft ionization techniques, such as electrospray ionization (ESI)^[4] and matrix-assisted laser desorption/ionization (MALDI),^[5] help ionize lipids in their native states, without requiring derivatization and without causing decomposition, thereby enabling the unequivocal determination of molecular weights. These soft ionization techniques are typically used in conjunction with tandem mass spectrometry (MS/MS) to provide structural detail and to help resolve isomers. Low energy collision-induced dissociation (CID) is the most prevalent MS/MS technique, and it has been employed for the structural analysis of a wide variety of lipid classes, including sphingomyelin (SM),^[6] phosphatidylglycerol (PG),^[7] glycerophosphoethanolamine (GPE),^[8] glycerophosphocholine (GPC),^[9] and glycerophosphatidic acid (GPA).^[10]

Low-energy collisional activation of lipids mainly produces fragments corresponding to the loss of entire fatty acyl substituents, like neutral ketenes and fatty acids, and is thus not informative enough for full structure characterization.^[11] To enhance the amount of structural information, a variety of MS/MS techniques have been explored as the alternative for the structural interrogation of lipids, including high-energy (HE) CID,^[12, 13] ion/molecule reactions such as Paternò-Büchi reactions,^[14] OzESI/OzID^[15–20], ion/ion reactions,^[21, 22] ion/photon reactions (e.g. UVPD,^[23] IRMPD^[24]), electron-based reactions (e.g. ETD,^[25] EIEIO,^[26] EID^[27, 28]) and radical-directed dissociation (RDD).^[29, 30]

In OzESI/OzID, the exposure of unsaturated lipids to ozone molecules results in an ozonide, which then dissociates into fragment ion pair(s) with diagnostic mass separation that enables an unambiguous identification of sites of unsaturation.^[16, 17] McLuckey, Blanksby and coworkers have shown that gas-phase ion/ion reactions can be used to convert lipid cations into their anion form, thereby providing incredible selectivity toward certain lipid classes.^[21, 22] When combined with low energy CID, ion/ion reactions could provide enhanced structural information, such as acyl chain lengths and degrees of unsaturation.^[21, 22] Jackson and coworkers have exploited the use of metastable atom-activated dissociation (MAD) mass spectrometry^[31] and MAD in combination with CID^[32] for the analysis of phospholipids. The high-energy radical-induced dissociation technique produced a range of fragments that are closely related to the number and location of double bonds in lipid acyl chains. Whereas the current state of the art in tandem mass spectrometry has a variety of approaches to target certain functional groups and chemistries, the communities interested in lipid characterization and lipidomics would stand to benefit from additional or complementary approaches to tandem mass spectrometry.

Charge transfer dissociation (CTD) is a possible alternative to the aforementioned MS/MS techniques, and it proceeds via exposure of gas-phase precursor cations to a kiloelectronvolt beam of helium cations.^[33] Upon the interaction with helium cations, peptide cations decompose via radical-driven pathways that are significantly different from low energy CID, but analogous to other high-energy fragmentation methods.^[33] CTD has also been demonstrated for complex oligosaccharides.^[34] CTD has the ability to increase the number of positive charges on a precursor ion and is workable with singly charged precursor ions, unlike ETD and ECD. The activation energy in CTD is determined by both the electron

affinity of the helium cation (~24.6 eV) and kinetic energy, and can drive reactions with appearance potentials greater than 24 eV.^[33, 34]

In this study, we demonstrate the utility of CTD as a means of structural characterization for phosphatidylcholines. Helium cation irradiation of protonated lipids produces highly extensive cleavage along lipid acyl chains (i.e. POPC, PSPC) and charge-increased ion series for lipids containing multiply carbon-carbon (C=C) double bonds (i.e. 9E- and 9Z-DOPC). The 12-Da peak spacing feature and the change in fragment ion intensity in the vicinity of the C=C double bond appear to be closely related to the position and geometry of C=C double bond(s), but with some questions remaining as to the exact mechanism of cleavage at the double bond position.

Experimental

Instrumentation

All mass spectra (CID, CTD and MAD) were collected on a Bruker amaZon ETD mass spectrometer (BrukerDaltronics, Bremen, Germany), which has been modified to perform lipid cation/helium cation or lipid cation/metastable atom reactions. Installation of saddle field fast ion/fast atom source (VSW/Atomtech, Macclesfield, UK), connection between electronic components and the working principle are highly analogous to our recent work on peptides and oligosaccharides.^[34, 35]

Materials

All the lipids used in this experiment were purchased from Avanti Polar Lipids (Alabaster, AL). The involved lipids and their shorthand designations are as follows: 1-hexadecanoyl-2-octadecanoyl-sn-glycero-3-phosphocholine (PSPC, 16:0/18:0), 1-hexadecanoyl-2-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine (POPC, 16:0/18:1(9Z)), 1,2-di-(9E-octadecenoyl)-sn-glycero-3-phosphocholine (9E-DOPC, 18:1/18:1(9E,9E)), 1,2-di-(9Z-octadecenoyl)-sn-glycero-3-phosphocholine (9Z-DOPC, 18:1/18:1(9Z, 9Z)), 1,2-di-(5Z,8Z,11Z,14Z-eicosatetraenoyl)-sn-glycero-3-phosphocholine (DAPC, 20:4/20:4), and sphingomyelin (SM, d18:1/18:0). Lipid analytes were prepared at a concentration of ~60 μ M in a solution of 49.5/49.5/1 (v/v/v) methanol/water/acetic acid prior to positive electrospray ionization (ESI).

Method

Each lipid solution was continuously infused into the ESI source with an electronic syringe pump (#1725, Hamilton Company Reno, Nevada, NV) at a flow rate of 160 μ L/h. The skimmer was at ground potential and the electrospray needle was set at 4.5 kV. The temperature of the heated capillary was 220°C. The $[M+H]^+$ or $[M+Na]^+$ ions were mass-selected using an isolation window of 1.0 or 4.0 Da depending on the need for isotope information. The saddle field ion source was only switched on during an MS² scan function in which the isolated ions were stored at a desired low mass cut-off (e.g. 150) with the excitation amplitude for CID set to zero volts. A 6 kV square wave with a pulse width of 25 ms was applied to the saddle field ion source for the generation of reagent helium cations. The helium flow was controlled via a variable leak valve, and the pressure read-out was

obtained from pressure monitor of the ion trap gauge in the main vacuum region. Using this indirect measurement, the helium gas supply was adjusted to provide a reading of $\sim 1.20 \times 10^{-5}$ mbar for all the experiments, which was barely above the base pressure of $\sim 8 \times 10^{-6}$ mbar in the main vacuum chamber. A typical low mass cut off (LMCO) value of m/z 150 was used for the removal of ionized residual background compounds. All the mass spectra (CID, CTD and MAD) were accumulated in profile mode with up to 4 minutes of averaging to improve the signal-to-noise ratio (S/N).

Results and Discussion

Helium cation irradiation of protonated POPC results in a range of fragments, as shown in Figure 1a. The CTD spectrum looks generally similar to the MAD spectrum (Figure 1b), but both differ greatly from traditional CID (Figure 1c)^[11] or electron ionization (EI) spectra^[36, 37]. All fragmentation methods give a dominant fragment ion at m/z 184.0, which is a diagnostic fragment of the phosphocoline head group.^[31, 38] The CTD spectrum also shows a doubly charged fragment at m/z 380.4, which corresponds to the charge-increased product $[\text{POPC}+\text{H}]^{2+}$, which is similar to the Penning ionized product ion observed in MAD.^[31] CTD shows three major fragments at m/z 478.4, m/z 496.4 and m/z 521.4, which are associated with entire acyl chain losses. These fragments resemble closely the MAD fragmentation pattern, but greatly differ from that of CID. Helium-CTD also shows an extensive dissociation along two acyl chains ranging from m/z 550 to m/z 732, which is also similar to MAD.

Helium-CTD of sodiated POPC produces a fragmentation pattern that highly resembles that of MAD spectrum of $[\text{POPC}+\text{Na}]^+$, as shown in Figure S1. In addition to the phosphocoline head group fragment at m/z 184.0 and the ionized species $[\text{M}+\text{Na}]^{2+}$ at m/z 391.5, CTD produced a variety of fragments related to cleavages of the glycerol backbone and its vicinity. Examples include the loss of trimethylamine ($\text{N}(\text{CH}_3)_3$) at m/z 723.5, the entire head group at m/z 599.5, sn-1/sn-2 acyl chains at m/z 526.5 and m/z 500.5, and simultaneous loss of two units, such as at m/z 441.4 and m/z 467.5. The cleavage of the C1-C2 bond within the glycerol backbone at m/z 513.5 was observed in the CTD and MAD spectra, but not CID. The CTD spectra of protonated POPC and sodiated POPC show different product ion distributions, as has been observed in low energy CID^[6, 9, 11] and post source decay (PSD) experiments.^[5] The distinction in PSD spectra for the two adduct forms is attributed to the different binding of H^+ and Na^+ to lipid head group.^[5]

The results in Figure 1 and S1 indicate that the CTD process involves both CID-like even-electron fragmentation pathways and MAD-like radical-induced fragmentation pathways.^[31] Ionization of 1+ precursor ions by Penning ionization or charge transfer are expected to be different in energy by the ionization potential of a helium metastable atom, which is about 4.77 eV.^[39] Although this difference seems significant, He metastable atoms and He cations are both more than 19.8 eV above the ground state, so both have more than enough energy to ionize most 1+ precursor ions, which typically have ionization energies of around 10 eV.^[40]

A more detailed comparison between CTD and MAD spectra of $[\text{POPC}+\text{H}]^+$ is given using the zoomed-ins in Figure 2a and 2b, and Figure 3a and 3b. In the region from m/z 470–540

(Figure 2a and 2b) the CTD spectrum of $[\text{POPC}+\text{H}]^+$ shows strong similarity to the MAD spectrum of the same lipid. The common features include neutral ketene losses at m/z 522 (sn-1) and m/z 496 (sn-2), as well as elimination of sn-2 fatty acid at m/z 478.^[31] Because the same batch of purchased POPC sample was used for both MAD and CTD experiments, the same set of contamination peaks at m/z 493.4 (loss of C(18:0) chain) and m/z 524.4 (loss of C(16:1) chain) were observed,^[31] possibly originating from the isomerization of POPC during its synthesis process.^[26, 41, 42] The spectrum in Figure 2d is a replicate experiment of Figure 2c, but with a much narrower isolation window (width = 1.0 Da). The exclusion of the ^{13}C contribution in Figure 2d helps to confirm the peak assignments in Figure 2c.

Different from the CID spectrum of PSPC (Figure S4b), the CTD spectrum in Figure 2c shows two sets of fragments associated with sn-1/sn-2 ketene losses: odd-electron fragments at m/z 495.5 and m/z 523.5, and even-electron fragments at m/z 496.5 and m/z 524.5. For both POPC and PSPC, the CTD spectra show preferential neutral ketene loss over neutral fatty acid loss, which resembles radical-induced EID^[28] but significantly differs from even-electron CID (Figure S4). Compared to the CTD spectra of PSPC and POPC in Figure 2,, the same even-electron fragments in the CID spectrum in Figure S4 are considerably less abundant. Hsu and Turk have shown that these same even electron ions can be enhanced in CID through lithiation of the lipids.^[10] The CID efficiency of lithiated precursors was sufficient to enable additional stages of CID to yield additional structural information.^[10]

For the even-electron fragments in Figure 2c, CTD does not show a distinctive preference in the formation of m/z 496 (sn-2 ketene loss) or m/z 522/524 (sn-1 ketene loss), which compromises its ability to differentiate between the sn-1 and sn-2 ketene losses. In this regard, CTD is slightly less informative than CID, which preferentially produces sn-2 ketene loss over sn-1 ketene loss (m/z 496 > m/z 522 or 524) (Figure S4).^[9, 28, 38] In contrast to this general preference for sn-2 ketene loss over sn-1 ketene loss, Ho and Huang show no such preference for sn-2 ketene loss in low energy CID experiments in a quadrupole ion trap^[11] and Jones et al. observed a slight preference for sn-1 ketene loss over sn-2 ketene loss in the EID fragmentation of phospholipids.^[28]

The odd-electron fragments in Figure 2c were also observed in MAD^[31] and EID^[28] spectra of the same lipid, which suggests analogous fragmentation pathways among CTD, MAD and EID. The odd-electron fragments must be generated via the introduction of radical species during the fragmentation process, indicating the involvement of radical cleavages in CTD.^[31] Interestingly, the fragment at m/z 495.5 (sn-2 position) is more abundant than the one at m/z 523.5 (sn-1 position). This trend agrees with that from the said EID results: the more favorable formation of radical cation associated with sn-2 position.^[28] This coincidence, along with the more favorable neutral ketene loss over fatty acid loss in CTD, is indicative of high resemblance of CTD in its mechanistic nature to that of EID.

For ease of discussion, the two spectra in Figure 3a and 3b are labeled in three sections; I, II, and III. The section labeled I shows the acyl cleavages close to the ω -end of the lipid chains. The CTD spectrum of $[\text{POPC}+\text{H}]^+$ (Figure 3b) shows extensive fragmentation along the two acyl chains in this region, including the even-electron rearrangement fragments at m/z 730.5, m/z 716.5, m/z 702.5, m/z 688.5, m/z 676.5 and m/z 662.5, which correspond to the neutral

loss of C_nH_{2n+2} molecules. In the same region, odd-electron fragments at m/z 731.5, m/z 717.5, m/z 703.5, m/z 689.5, m/z 675.5 and m/z 661.5 are also observed (green font), which corresponding to neutral loss of $C_nH_{2n+1}^{\bullet}$ alkyl radicals. Almost the same even-/odd-electron fragment series were observed in both MAD (Figure 3a) and EID spectra reported elsewhere.^[28]

The ladder-like patterns of even-electron fragments are separated by 14.0 Da, which is a commonly observed pattern in EI^[36, 37] and HE-CID,^[43] as well as the recently reported electron-based MS/MS experiments on lipids, EIEIO^[26] and EID.^[28] The accompanying odd-electron fragments associated with the losses of alkyl radicals were also reported in other high-energy MS/MS experiments.^[26, 28, 43] Others have proposed that the serial neutral loss of C_nH_{2n+2} could either be the neutral loss of an alkane or be the neutral loss of an alkene+H₂ (i.e. 1,4-cyclic elimination).^[28, 44] In most general respects, CTD fragmentation can therefore be rationalized through radical mechanisms proposed by others.^[43, 45]

In section II, the vinyl bond vicinity, CTD-generated fragments exhibit identical nominal masses to that of MAD, but some notable differences were also observed between MAD and CTD. For example, MAD shows reduced intensities at the C=C double bond site along with the elevated ion intensity corresponding to distal allyl cleavages—the most prevalent dissociation pattern of unsaturated acyl chains, which has been widely reported in FAB,^[46] HE CID,^[45] EIEIO,^[26] and EID^[28] experiments. In CTD, this vicinity looks slightly different and contains a distinctive pair of peaks at m/z 620.5 and m/z 632.5, whose spacing is a diagnostic 12 Da. This characteristic peak spacing has been well studied and documented as the diagnostic value for localization of C=C double bonds. Mass spectrometric experiments involving EI,^[47] HE-CID,^[48] RDD^[29, 30] and MAD-MS³ CID^[32] have made use of this diagnostic feature for the determination of double bond positioning in unsaturated fatty acid derivatives and phospholipids. Aside from the above radical-directed fragmentation techniques, the localization of double bond(s) in lipid acyl chains has also been made possible using low energy CID experiments,^[10] although the methodology is completely different from the above. Monolithiated and dilithiated glycerophospholipids were subjected to multiple-stage collisional activation (MSⁿ, n=3,4), which offered information for pinpointing double-bond location along fatty acyl substituents.

Similar to MAD, CTD only produces a few fragments in section III, the α -end of the acyl chain, including contributions from both sn-1 and sn-2 acyl chain cleavages. It is generally rare to observe dissociation in this region of the lipid, but the fragments observed for CTD are analogous to the recently reported EID results of [POPC+H]⁺.^[28] The fragments at m/z 577.6, and m/z 576.5 presumably result from cleavage related to head group loss.

Consistent with CTD results of POPC, CTD of PSPC (Figure 3c) also produces extensive dissociation along two acyl chains, with an even greater extent of fragmentation. PSPC is structurally different from POPC in that it contains two fully saturated acyl chains (16:0/18:0). Consequently, a more extensive ladder-like dissociation pattern can be seen from m/z 718.6 to m/z 550.5, which corresponds to the mutual contribution of sn-1 and sn-2

acyl chains. Moreover, the fragment ion intensities appear to be more uniform along the entire saturated acyl chains.^[26] It is worth noting that the nominal masses from m/z 592.5 to m/z 718.6 are in one-to-one correspondence with those in EID of PSPC.^[28] A difference between POPC and PSPC is that CTD of PSPC does not produce the aforementioned odd-electron fragment series. The lack of odd-electron fragments in PSPC is analogous to EID experiments.^[28] For PSPC, the loss of the head group at m/z 578.5, appears more abundant than for POPC, but the difference in abundance relative to the acyl cleavages is due to decrease in the abundance of acyl fragments for PSPC, not an increase in the abundance of the head group loss.^[28]

CID and CTD spectra of protonated 9E-DOPC (18:1/18:1) are shown in Figure 4a and 4b. Collisional activation of this lipid only produces three fragments, as we reported before.^[31] However, CTD of the same lipid produces a much more extensive fragmentation coverage than CID, which includes head group losses at m/z 184.0, m/z 521 for the sn-1/sn-2 alkyl ketene loss and m/z 505 for the sn-1/sn-2 fatty acid loss. CTD also produces charge-increased, or oxidized, product ions such as $[9E-DOPC+H]^{2+}$ at m/z 393.5, $[9E-DOPC+H-C_9H_{19}]^{2+}$ at m/z 330.5, and acyl chain cleavages at the C=C double bond position. This pattern is almost identical to a previously reported MAD spectrum of $[9E-DOPC+H]^+$ (18:1/18:1).^[31] The close similarity between MAD and CTD suggests a similar mechanistic nature between them, although the mechanism of cleavage at the double bond position has not yet been elucidated.

The middle panels (c and d) of Figure 4 compare the range m/z 500–530 for CID and CTD results of 9E-DOPC. The bottom panels of Figure 4(e and f) show CID and CTD spectra of 9Z-DOPC. In CTD, the peak patterns around m/z 521 and m/z 505 resemble MAD spectra of the same species,^[31] but vastly differ from that of CID. CID mainly proceeds through even-electron rearrangements thus yielding even-electron fragments. The zoomed-in CTD spectrum of cis-double bond lipid (9Z-DOPC) looks very similar to that of trans-double bond lipid (9E-DOPC), which agrees with the reported difficulty in differentiating cis- and trans-geometry of double bonds.^[49] For CTD of both 9E- and 9Z-DOPC, the preference in ketene loss (m/z 522.5) over fatty acid loss (m/z 506.5) is in contrast to CID spectra. This preferential ketene loss in CTD is consistent with the aforesaid trend for POPC and PSPC. The reproducible feature across lipids with different acyl chain combinations further confirms the distinctive mechanistic nature of CTD, which should be different from that of even-electron CID, but is close to radical dissociation feature of MAD, EIEIO and EID.

Figure 5a and 5b show magnified CTD spectra of $[9E-DOPC+H]^+$ and $[9Z-DOPC+H]^+$ from m/z 530–750. In contrast to PSPC, which has two saturated acyl chains, 9E- and 9Z-DOPC both contain two unsaturated acyl chains. Consistent with CTD results of POPC and PSPC, a ladder-like fragmentation pattern was observed in CTD spectra of both 9E- and 9Z-DOPC. Different from the CTD results of POPC and PSPC, fewer fragments were observed for 9E- and 9Z-DOPC. The lack of CC single bond cleavages closer to ω -end was also seen in EID spectra of 9Z- and 6Z-DOPC.^[28] It seems that the presence of multiple C=C double bonds obstructs the propensity of fragmentation in CTD and other radical-induced approaches.

Consistent with the CTD results of POPC, the diagnostic peak spacing of 12 Da was also observed in the double bond region for both 9E- and 9Z-DOPC, which offers an unambiguous localization of C=C double bonds in both lipids. The consistency in this 12 Da spacing demonstrates the reproducibility of CTD in producing this double bond-specific feature. These results also indicate the promising potential of CTD for the diagnosis or differentiation of sites of unsaturation in lipids, or further possible extension into other biomolecules with unsaturated olefinic chains, such as fatty acids methyl esters (FAMES).

In contrast to the CTD spectra of POPC and PSPC, CTD spectra of 9E- and 9Z-DOPC show a unique neutral losses, including: m/z 508 ($-C_{20}H_{38}$), m/z 522 ($-C_{19}H_{36}$), m/z 536 ($-C_{18}H_{34}$), m/z 550 ($-C_{17}H_{32}$), m/z 564 ($-C_{16}H_{30}$) and m/z 578 ($-C_{15}H_{28}$) (orange font in Figure 4c, 4d, 5a and 5b). The tentative assignments are shown in parentheses, above. This type of C_nH_{2n-2} neutral loss is consistent with the observation in EID experiments, which could be attributed to the mutual cleavages of both unsaturated acyl chains.^[28]

Figure 6 compares the unique doubly charged ion series in CTD of $[9E-DOPC+H]^+$ and $[9Z-DOPC+H]^+$, which shows a peak spacing of 7.0 Da instead of 14.0 Da. To our best knowledge, doubly charged product ions showing a 7.0 Da-ladder pattern is rarely reported in gas-phase ion activation experiments. Nevertheless, this pattern was also observed in MAD spectra of 9E- and 9Z-DOPC,^[31] again suggesting a significant mechanistic similarity between CTD and MAD. The doubly charged ion series almost covers the entire acyl chain. 9E- and 9Z-DOPC spectra exhibit a similar extent of chain cleavage, and also show a similar fragment ion intensity distribution. Noticeably, the most abundant peaks are at m/z 330 and m/z 337 corresponding to cleavages at or next to the site of unsaturation in both lipids.

In the spectrum for 9E-DOPC (Figure 6a), the peak at m/z 330 is more abundant than m/z 337; while in 9Z-DOPC spectrum, the trend appears to be reversed. The variation in fragment ion intensities was reproducible on different days and is therefore more likely to be sensitive to the geometry of double bond rather than experimental irreproducibility. Because 9E- and 9Z-DOPC only differ in double bond geometry, identical fragment masses are generated for both lipids. Given the similar dissociation pattern and lack of diagnostic fragments, the most common way to discriminate them is to track the changes in relative abundances of certain fragments. This concept has been reported and utilized in the differentiation of geometrical isomers of FAMES using low-energy electron ionization mass spectrometry.^[49]

Figure 7 shows the comparison between CTD and CID spectra of protonated sphingomyelin. Collisional activation of sphingomyelin produces very few fragments: m/z 184.0, associated with phosphocholine head group loss, and m/z 713.6, associated with a neutral water loss.^[31] The inefficiency of CID in producing structurally informative fragments is consistent with literature reports.^[6, 46, 50]

However, CTD of the same precursor of sphingomyelin (SM) is capable of producing a few additional fragments, including a characteristic charge-increased product ion ($[M+H]^{2+}$) at m/z 365.9 and two fragments at m/z 447.4 and m/z 491.4 corresponding to the entire acyl chain losses. These distinctive product ions are only observed in CTD and not observed in

MAD of the same species.^[31] Sphingomyelin is structurally different from the other tested phospholipids: one fatty acyl group is alkylated to the lipid backbone, with the other fatty acyl group being connected to sphingosine via an amide bond.^[46] The absence of the two ester-connections could possibly make a less “fragile” molecule, resulting in a less efficient dissociation pattern of CID^[6], MAD and CTD experiments. However, exceptions do exist. Baba and coworkers reported a highly efficient fragmentation upon the SM class using electron impact excitation of ions from organics (EIEIO)/electron-induced dissociation (EID)^[51]. In addition to the lipid class information, their experiments were able to provide information regarding acyl group structure and the double bond(s) positioning for nearly 200 SM molecules.

CID and CTD spectra of $[\text{DAPC}+\text{H}]^+$ are shown in Figure S5. Upon collisional activation, $[\text{DAPC}+\text{H}]^+$ mainly produces fragments corresponding to head group loss, sn-1/sn-2 fatty acid and alkyl ketene losses, which is quite similar to the pattern of CID of $[\text{9E-/9Z-DOPC}+\text{H}]^+$. CTD of DAPC produces the same cleavages, but also produces 1+ and 2+ fragments in the vicinity of the four double bonds. The charge-increased product ion $[\text{M}+\text{H}]^{2+}$ at m/z 415.4 was generated, as was the case for all the examined phospholipids. The zoomed-in region from m/z 500 to 850 shows a relatively poor S/N ratio, which is inferior to that of MAD spectrum.^[31] Consistent with MAD of DAPC, CTD of DAPC also produces quite limited cleavages, presumably because of the extra resonance stability afforded by the numerous double bonds.^[31]

Conclusions

Charge transfer dissociation mass spectrometry (CTD-MS) has previously been shown as a promising alternative for structure interrogation of gas-phase peptide ions and complex carbohydrates.^[34] Herein, we report CTD-MS on a different set of biomolecules—phospholipids—gives rise to helpful CID-like fragments, but also produces extensive dissociation within lipid acyl chains, which provides information that is not achievable through CID. The additional structural information includes the C=C double bond positioning, and possible the stereochemistry. Importantly, the diagnostic spacing of ion pairs is preserved across a range of lipids with varying acyl chain lengths and number of C=C double bonds. If tested on a larger pool of lipids, CTD could be exploited to probe the structure of other classes of lipids or to the gas phase chemistry of other biomolecules.

The quite poor signal to noise and spectral complexity make CTD less appealing than OzID or other radical-induced methods, but improvements in efficiency and signal to noise could make CTD more appealing for lipid analyses in the future. For the lipids studied, CTD and MAD provide very similar fragmentation patterns and efficiencies, with neither technique having clear benefits over the other. In certain applications, it may be beneficial to have a beam of neutral reagent atoms that are unaffected by electric fields. In other applications, one may benefit from using the helium cation beam of CTD to focus or steer the beam as desired.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors acknowledge financial support from the National Institutes of Health (NIH) (1R01GM114494-01). The opinions, findings, and conclusions or recommendations expressed in this publication are those of the author(s) and do not necessarily reflect the views of NIH.

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Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.

(a) SM(d18:1/18:0) **(b) SM(d18:1/18:0)** **Figure 7.**