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Rapid characterization of the fatty acyl composition of complex lipids by collision-induced dissociation time-of-flight mass spectrometry

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Abstract Profiling of leaf extracts from mutants of *Arabidopsis* with defects in lipid desaturation demonstrates the utility of collision-induced dissociation time-of-flight mass spectrometry (CID-TOF MS) for screening biological samples for fatty acid compositional alterations. CID-TOF MS uses the collision cell of a quadrupole time-of-flight mass spectrometer to simultaneously fragment all of the ions produced by an ionization source. Electrospray ionization CID-TOF MS in the negative mode can be used to analyze fatty acyl anions derived from complex lipids as well as free fatty acids. Although acyl anion yield is shown to be a function of the lipid class and the position on the glycerol backbone, acyl compositional profiles can be determined, and the TOF detector provides resolution of nominally isobaric acyl species in the profiles. Good precision is obtained when data are acquired for ~1 min per sample.—Esch, S. W., P. Tamura, A. A. Sparks, M. R. Roth, S. P. Devaiah, E. Heinz, X. Wang, T. D. Williams, and R. Welti. Rapid characterization of the fatty acyl composition of complex lipids by collision-induced dissociation time-of-flight mass spectrometry. *J. Lipid Res.* 2007. 48: 235–241.

Supplementary key words phospholipid • galactolipid • oxylipins • lipidomics

Fatty acyl composition is most commonly analyzed by gas chromatography after derivatization to fatty acid methyl esters. When flame ionization or a mass spectrometer is used as the detector, quantitative compositional data are obtained. Liquid chromatography provides an alternative means of separation, in which fatty acids can be detected by ultraviolet light absorption after chemical conversion to an appropriate derivative form (1). Separation and analyti-

cal techniques include adsorption chromatography, silver nitrate chromatography, TLC, and HPLC (2). Most gas chromatography and liquid chromatography protocols for fatty acyl species are optimized for normal chain fatty acyl species. Other fatty acids, such as those containing heteroatoms (e.g., oxygen), often require specialized procedures for analysis. Plant oxylipins, for example, have been analyzed by GC-MS or liquid chromatography after various extraction and derivatization steps designed to enable detection and minimize the degradation of these often low-level compounds (3–7).

Recently, we used a novel negative ion mode mass spectrometric scanning method for rapid profiling of free and ester-linked fatty acyl moieties in crude lipid extracts (8). The collision-induced dissociation time-of-flight (CID-TOF) experiment involved simultaneous collision-induced dissociation of all members of a complex mixture of lipids in the collision cell of a quadrupole time-of-flight (Q-TOF) instrument. The TOF analyzer provided accurate mass characterization of all intact acyl fragments in a single spectrum. Using this method as a “discovery scan,” we identified fatty acyl groups, including several oxygenated species that increased in response to wounding of the plant *Arabidopsis thaliana* (8). Here, we describe this methodology and investigate the accuracy and precision of collision-induced dissociation time-of-flight mass spectrometry (CID-TOF MS).

Abbreviations: CID-TOF MS, collision-induced dissociation time-of-flight mass spectrometry; di15:0 PE, 1-15:0,2-15:0 phosphatidylethanolamine; ESI, electrospray ionization; FID, flame ionization detection; MGDG, monogalactosyldiacylglycerol; OPPC, 1-oleoyl (18:1),2-palmitoyl (16:0) phosphatidylcholine; POPC, 1-palmitoyl (16:0),2-oleoyl (18:1) phosphatidylcholine; POPE, 1-palmitoyl (16:0),2-oleoyl (18:1) phosphatidylethanolamine; Q-TOF, quadrupole time-of-flight.

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Lipids

1-15:0,2-15:0 phosphatidylethanolamine (di15:0 PE), POPC, 1-oleoyl (18:1),2-palmitoyl (16:0) phosphatidylcholine (OPPC), and 1-palmitoyl (16:0),2-oleoyl (18:1) phosphatidylethanolamine (POPE) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). 17:0 fatty acid was purchased from NuChek Prep, Inc. (Elysian, MN). 1-18:1,2-16:1 monogalactosyldiacylglycerol (MGDG) was prepared semisynthetically (9).

Plant extracts

Leaves from approximately 7 week old *Arabidopsis* (Columbia ecotype) plants were used for analysis. Growth conditions have been described elsewhere (8). Wounding was performed as described previously (8, 10). *Arabidopsis* lipids were extracted as described previously (11). Seeds of the *act1-1*, *fad2-1*, *fad3-2*, and *fad7-1(gl1)* mutants of *Arabidopsis* were obtained from the Arabidopsis Biological Resource Center at Ohio State University (catalog Nos. CS200, CS8041, CS8034, and CD3108, respectively). Because a high-throughput analytical approach is being developed, extracts were analyzed in crude form, without further processing, except as noted. Crude extracts containing the lipid from ~2 mg leaf biomass (dry weight)/ml chloroform were diluted 100-fold into the infusion solvent before mass analysis.

For purposes of analyzing collision energy effects only, a crude extract of 7 week old *Arabidopsis* (Columbia ecotype) was further processed to reduce levels of naturally occurring free fatty acids. Crude extract (500 μ l in chloroform) was passed through an activated silicic acid (Unisil; Clarkson Chemical Co., Williamsport, PA) column (0.3 g suspended in chloroform and packed into a glass Pasteur pipette) and washed with 8 ml of chloroform to elute the neutral lipids. Polar lipids were then eluted with 8 ml of chloroform-methanol (2:1), the solvent was evaporated to dryness, and the lipids were dissolved in 1 ml of chloroform. This, in turn, was diluted ~100-fold into the electrospray solvent before mass analysis.

CID-TOF MS

Electrospray ionization (ESI)-CID-TOF MS spectra were acquired with a Micromass Q-TOF-2 tandem mass spectrometer (Micromass, Ltd., Manchester, UK). The TOF analyzer was tuned for maximum resolution (10,000 resolving power) with argon in the collision cell. The pusher frequency was set at 22 kHz for a mass range of m/z 20–1,000 and at 16 kHz for a mass range of m/z 20–2,000. Mass selection in the Q1 quadrupole was turned off, whereas the collision cell remained at the specified voltage offset. Unselected precursor ions were subjected to CID in the negative mode. Micromass MassLynx software was used as the operating software. All TOF spectra were acquired with daily mass calibration. Samples were dissolved in chloroform-methanol-300 mM ammonium acetate in water (60:133:7, v/v/v) and infused into the ESI source of the Q-TOF at 20–40 μ l/min.

CID-TOF MS data analysis

Careful selection of background subtraction, smoothing, and centroiding parameters was required for reproducible processing of CID-TOF raw continuum spectral data. Spectrum manipulation software was used to attenuate chemical background noise to ~10 counts per ion by setting a linear horizontal baseline subtraction threshold to remove the lowest 40% of signals in the continuum spectrum. After baseline subtraction, the spectral data were subjected to two successive smooths of ± 3 "time bin" channels (0.0625 u/channel) according to the Savitzky-Golay method

(12). Smoothed peaks were centroided at a minimum peak width at half height of four channels (0.25 units). These manipulations were adjusted by trial and error to ensure one centroid "stick" per resolvable ion in this region. Spectra were mass-corrected by locking on an appropriate known anion in the m/z region of interest. Chemical formulas for the product ions were determined using the Micromass MassLynx chemical formula tool limiting formula to C, H, N, O, P, S, sensible valence, and rings plus double bonds restrictions.

For calculation of fatty acid composition, the centroided, mass-corrected spectral data were transferred to an Excel data template with a "lookup" function that selected mass/signal pairs within specified m/z windows for the expected and identified acyl anions. The intensities of acyl-specific signals were summed, divided by the total signal, and multiplied by 100 to obtain mol% of total fatty acids. It was not necessary to correct for A + 2 isotopic overlap, because in no case did the m/z of the A + 2 peak of an examined fatty acid fall within the m/z window of the A peak of another fatty acid isotope cluster.

Gas chromatography with flame ionization detection

The solvent was completely evaporated from samples to which an internal standard of 15:0 had been added. One milliliter of 3 M methanolic hydrochloric acid was added, and the samples were bubbled with nitrogen gas and heated at 78°C for 30 min. Two milliliters of water and 2 ml of pentane were added. Samples were shaken, and the upper pentane layer was removed. This extraction was repeated twice, the solvent was evaporated, and the sample was dissolved in 150 μ l of hexane.

Samples were analyzed by gas chromatography with flame ionization detection (FID) on an Agilent 6890 gas chromatograph (Agilent Technologies, Wilmington, DE) equipped with a split/splitless injector operated in the splitless mode at 250°C. The injection volume was 1 μ l. The carrier gas was helium (0.7 ml/min). Compounds were separated on a Supelco 2380 [stabilized; poly(90%:10% biscyanopropyl-cyanopropylphenyl siloxane)] column (30 m \times 0.25 mm inner diameter; Sigma-Aldrich, St. Louis, MO). The column temperature was ramped from 50°C to 130°C at 20°C/min, then to 250°C at 3°C/min. The FID detector was at 260°C.

RESULTS AND DISCUSSION

CID-TOF MS detects acyl anions from complex lipids

Fatty acyl compositional analysis by CID-TOF MS is performed by ionizing a solution of complex lipids by ESI Q-TOF MS in negative ion mode with ammonium acetate as carrier electrolyte, producing $[M-H]^-$ and/or $[M+OAc]^-$ acyl anions. The quadrupole is operated in radio frequency-only mode (no direct current voltage applied), allowing all lipid analytes supplied by the ion source entry into the collision cell simultaneously, regardless of size (i.e., m/z value). The acyl anions are detected by a TOF analyzer. **Figure 1** shows the result of CID-TOF MS analysis performed at three different collision energies (10, 35, and 60 eV) on an extract of *Arabidopsis* leaves after silicic acid chromatography to reduce the free fatty acid levels in the extract. These spectra were obtained by direct infusion of the lipid extract and, as shown, are each a combination of 11 scans of 5 s duration of m/z 20–2,000.

When the voltage offset on the collision cell was 10 eV (Fig. 1A), only very low levels of acyl anions could be

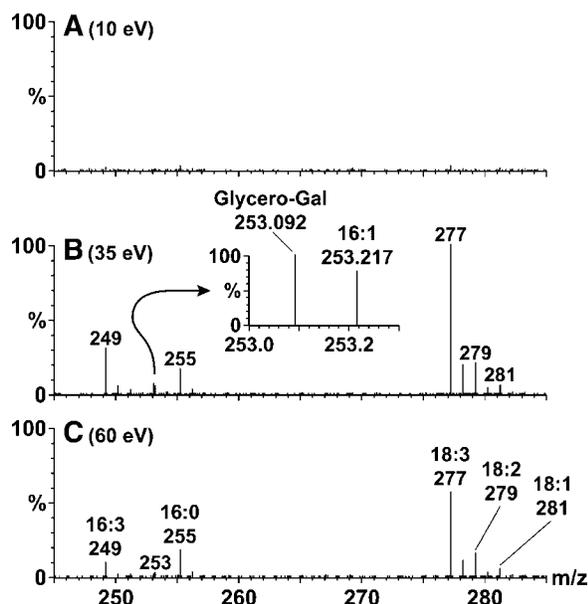


Fig. 1. Effect of collision energy on acyl yield in collision-induced dissociation time-of-flight mass spectrometry (CID-TOF MS) of plant lipids. Centroided CID-TOF analysis of column-purified polar lipids from wild-type *Arabidopsis* at collision energies of 10, 35, and 60 eV (spectra A, B, and C, respectively) show that total and relative anion yields change as a complex function of collision energy. All measured intensities are plotted relative to the base peak intensity for the 18:3 acyl anion at 35 eV (m/z 277). The most abundant acyl anions are labeled in C. A mass resolution slightly $>6,000$ is more than sufficient to achieve baseline separation of peaks for the glycerogalactose fragment of the monogalactosyldiacylglycerol (MGDG) head group (m/z 253.0923) and the nominally isobaric 16:1 acyl anion (m/z 253.2168), as shown in the inset for spectrum B (centroided data). Tiny acyl anion peaks in the 10 eV data may have been attributable to autolysis of the sample after column purification, because no expected lipid head group fragments appeared above the limits of detection to indicate that fragmentation had occurred in the collision cell at 10 eV. The acyl anion for 18:3 was chosen as the lock mass for all spectra, and each spectrum is the sum of 11 scans of 5 s duration.

detected in this sample lacking free fatty acids. However, when higher collision offset voltages were applied to the collision cell, negative anions were readily produced and detected. The spectra obtained at 35 and 60 eV (Fig. 1B, C) show abundant acyl anions as well as characteristic head group-derived fragments. These data indicate that the acyl anions observed (Fig. 1B, C) arise from CID of the complex lipids. The accurate mass capability of the TOF analyzer makes it possible to differentiate between nominally isobaric species (13, 14). For example, as shown in the inset to Fig. 1B, the spectral peak at m/z 253.092, corresponding to the chemical formula $C_9H_{17}O_8$, which is a glycerogalactose fragment derived from MGDG, is resolved from the peak observed at m/z 253.217, corresponding to the chemical formula $C_{16}H_{29}O_2$, which is a 16:1 acyl anion. Compared with the 35 eV spectrum (Fig. 1B), the spectrum obtained at 60 eV (Fig. 1C) has somewhat less total ion current (61%) in the peaks in the m/z range of interest.

CID-TOF MS fatty acyl analysis is not rigorously quantitative

Although the spectra in Fig. 1B, C are similar, the relative heights of acyl anion peaks differ in response to fragmentation at different collision energies. In **Table 1**, these acyl anions are quantified and compared with the fatty acyl composition determined by fatty acid methyl ester analysis of the same *Arabidopsis* lipid mixture by GC with FID. By GC-FID, the detector response varies by $<10\%$ among the analyzed fatty acid methyl esters (15, 16), so GC-FID data indicate a composition close to the true composition. The spectral data produce a composition similar to, but not exactly the same as, the fatty acyl composition determined by GC-FID. Furthermore, the composition determined by CID-TOF depends somewhat on the offset voltage applied to the collision cell.

Polar lipids of known composition and concentration were used to evaluate the influence of lipid structure on acyl anion peak yields in CID-TOF MS spectra collected at 35 eV (**Fig. 2**). Peak size was influenced by both the nature of the polar head group and the acyl chain position on the glycerol backbone. Figure 2A shows the 35 eV CID-TOF MS spectrum derived from a 1:1:1 (molar basis) mixture of POPC, di15:0 PE, and 17:0 fatty acid. The size of the acyl peak derived from POPC relative to the size of the 15:0 peak from di15:0 PE was 0.65:1 [(16:0 + 18:1 peak intensities)/15:0 peak intensity], as shown in **Table 2**. Figure 2B shows the spectrum obtained under identical conditions with OPPC (same head group and acyl composition as the PC complex lipid in Fig. 2A but with the *sn1/sn2* acyl positions reversed), di15:0 PE, and 17:0 fatty acid. The size of the peak derived from OPPC relative to the peak from di15:0 PE was 0.64:1, almost the same as in Fig. 2A. As might be expected of complex lipids with the same head group, the relative sizes of the acyl anion peaks produced from POPE and di15:0 PE were $\sim 1:1$ (Fig. 2C). The relative sizes of the MGDG and di15:0 PE acyl anion peaks were also $\sim 1:1$ (Fig. 2D). Table 2 details these relative

TABLE 1. Fatty acid quantitation by CID-TOF versus GC-FID

Acyl Chain	CID-TOF		GC-FID
	35 eV	60 eV	Fatty Acid Methyl Ester
	<i>mol%</i>		
16:3	17.1	8.8	14.5 \pm 0.1
16:2	1.1	1.0	0.9 \pm 0.0
16:1	2.9	2.3	4.2 \pm 0.0 ^a
16:0	9.2	16.6	14.4 \pm 0.1
18:3	56.1	52.8	45.7 \pm 0.2
18:2	10.2	13.2	15.6 \pm 0.0
18:1	3.0	4.6	3.4 \pm 0.0
18:0	0.4	0.7	1.2 \pm 0.1

CID-TOF, collision-induced dissociation time-of-flight; GC-FID, gas chromatography with flame ionization detection. Aliquots of unwounded wild-type *Arabidopsis* polar lipids were analyzed by CID-TOF at 35 and 60 eV. Three aliquots were used to prepare fatty acid methyl esters. GC-FID data are means \pm SD ($n = 3$). Amounts in mol% were normalized to the total amount detected for the target acyl groups in each approach.

^aGC-FID resolved 16:1 with a 7,8-*cis* double bond (0.8%) from that with a 3,4-*trans* double bond (3.4%).

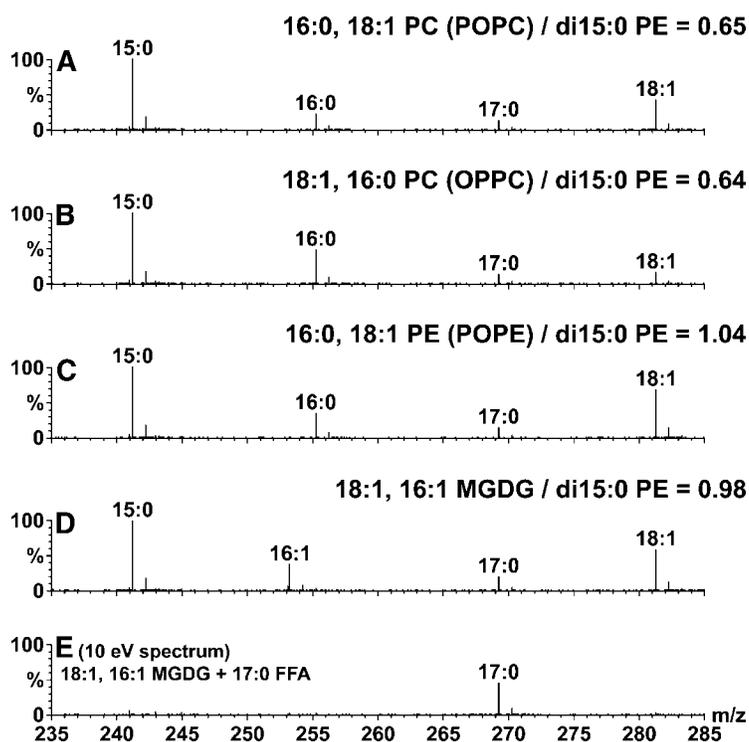


Fig. 2. Effect of polar head group and position on the glycerol backbone on acyl yield via CID-TOF. Each spectrum represents 11 scans of 5 s duration, and all spectra were acquired at 35 eV except for spectrum E, which was acquired at 10 eV. Each panel depicts a centroided CID-TOF spectrum of an equimolar mixture of 15:0, 15:0 phosphatidylethanolamine (di15:0 PE), 17:0 free fatty acid, and an additional synthetic lipid. The additional lipid in spectrum A is POPC; that in spectrum B is 1-oleoyl (18:1),2-palmitoyl (16:0) phosphatidylcholine (OPPC); that in spectrum C is 1-palmitoyl (16:0),2-oleoyl (18:1) phosphatidylethanolamine (POPE); that in spectra D and E is 1-18:1,2-16:1 MGDG. The 17:0 acyl anion was used as the common lock mass, and correction for isotopic effects was approximated by summing the monoisotopic, A + 1, and A + 2 isotope peak intensities. Resulting data for spectra A through D are summarized in Table 2, together with corresponding data taken at 60 eV and processed in a similar manner.

intensities and shows that at 35 eV collision energy, complex lipids containing PE and MGDG head groups ionized and/or fragmented to approximately the same level, and to a greater extent than lipids containing PC. The relative propensity of PE and PC lipids to produce acyl anions was reversed at 60 eV, however, at which voltage the ratios of POPC and OPPC to di15:0 PE were both 1.25. At 60 eV,

MGDG also ionized somewhat more efficiently than the PE species.

The variability of acyl anion yield from different head groups may have resulted from different kinds of precursor ion formation arising during ionization in negative mode. For example, in chloroform-methanol-aqueous 300 mM NH_4OAc (60:133:7, v/v/v), ESI of PC produces

TABLE 2. Acyl chain intensity data for mixed lipid standards at 35 and 60 eV

Trial	Acyl Group	35 eV Collision Energy			60 eV Collision Energy		
		Parent Lipid	Percent Total Acyl Intensity	Percent Total Lipid Intensity	Parent Lipid	Percent Total Acyl Intensity	Percent Total Lipid Intensity
A	16:0	POPC	12.3	36.6	POPC	19.6	52.1
	18:1	POPC	24.4		POPC	32.5	
	15:0	di15:0 PE	56.2	56.2	di15:0 PE	41.6	41.6
	17:0	Free acid	7.1	7.1	Free acid	6.3	6.3
B	18:1	OPPC	8.9	36.0	OPPC	15.9	52.2
	16:0	OPPC	27.1		OPPC	36.4	
	15:0	di15:0 PE	56.6	56.6	di15:0 PE	41.6	41.6
	17:0	Free acid	7.3	7.3	Free acid	6.2	6.2
C	16:0	POPE	15.7	47.8	POPE	19.6	52.2
	18:1	POPE	32.1		POPE	32.6	
	15:0	di15:0 PE	45.9	45.9	di15:0 PE	40.3	40.3
	17:0	Free acid	6.3	6.3	Free acid	7.5	7.5
D	18:1	MGDG	27.9	45.1	MGDG	30.1	47.5
	16:1	MGDG	17.2		MGDG	17.4	
	15:0	di15:0 PE	46.0	46.0	di15:0 PE	40.8	40.8
	17:0	Free acid	9.0	9.0	Free acid	11.7	11.7

di15:0 PE, 1-15:0,2-15:0 phosphatidylethanolamine; MGDG, monogalactosyldiacylglycerol; OPPC, 1-oleoyl (18:1),2-palmitoyl (16:0) phosphatidylcholine; POPE, 1-palmitoyl (16:0),2-oleoyl (18:1) phosphatidylethanolamine. Acyl anion intensity data were normalized to the total intensity for all four acyl groups targeted in each CID-TOF experimental trial. Equimolar mixtures of a single target lipid, di15:0 PE, and 17:0 free fatty acid were evaluated. Structures of target lipids POPC, OPPC, POPE, and 18:1,16:1 MGDG were as described in the legend for Fig. 2. The acyl group at the *sn1* position of each target lipid is listed first. Percent total lipid intensity for target lipids is reported as the arithmetic sum of the measured *sn1* and *sn2* acyl intensities.

primarily $[M+OAc]^-$ ions and, to a lesser extent, $[M-CH_3]^-$ ions, PE produces primarily $[M-H]^-$ ions, and MGDG produces both $[M+OAc]^-$ and $[M-H]^-$ ions. Therefore, given the potential for variability in the type of precursors formed, it is perhaps remarkable that at both electrospray voltages examined, the PCs, PE, and MGDG produced acyl anions with relative intensities differing by less than a factor of 1.5. Thus, acyl anions derived from the various head group classes in a biological mixture could all be expected to contribute similarly to the spectrum. The differences in signal produced by PC species compared with PE and MGDG species in this experiment, however, imply that no single collision energy is optimal for the quantitative recovery of acyl groups from different lipid classes.

The data in Fig. 2 also indicate that the acyl chain position affects the CID-TOF MS spectral signal. For example, for POPC (Fig. 2A), the ratio of the peak produced by the *sn2* acyl species compared with the *sn1* acyl species (18:1 to 16:0) was 2.0, demonstrating a higher yield from the 2-position (Table 2). Similarly, OPPC and POPE also yielded more acyl anion from the 2-position (Fig. 2B, C, Table 2). In each of these three phospholipid cases, the more abundant acyl yield was obtained from the *sn2* position, confirming observations of product ion analysis described by Murphy (17). The *sn2:sn1* ratios were not identical, however, suggesting that acyl chain structure could play a secondary role in the CID-TOF yield obtained. On the other hand, the acyl yields from the 1- and 2-positions of the MGDG lipid were reversed, and the fatty acyl group in the 1-position gave a higher yield (Fig. 2D, Table 2). Guella, Frassanito, and Mancini (18) also found that fragmentation of MGDG to produce acyl anions generally occurred more readily from the *sn1* than the *sn2* position. These results allow us to resolve the ambiguity in a previous study (19) in which the acyl positions in naturally occurring 18:3-16:3 MGDG and 18:3-16:3 digalactosyldiacylglycerol were reported provisionally as 16:3-18:3, based on the higher yield of 18:3 acyl anion and the (incorrect) assignment of this higher-yield anion to the species in the 2-position.

The 17:0 free fatty acid, included in all of the samples in Fig. 2, was the only acyl anion recovered in substantial yield at 10 eV (Fig. 2E). This confirms the observation that MS1 spectra or spectra obtained at very low collision energies (those minimally required to usher intact ions through the collision cell) are useful for examining background levels of free fatty acids in tissue extracts (20). It should be noted that the yield of the 17:0 fatty acid was somewhat lower at 35 eV (Fig. 2A–D) than at 10 eV (all panels are on the same scale). This may reflect fragmentation by CID of the 17:0 fatty acyl anion at 35 eV, at which voltage the ratio of 17:0 to di15:0 PE is <1:1, the ratio that would be expected for the free fatty acid relative to the same molar amount of PE (Table 2).

Together, the data in Fig. 2 and Table 2 indicate that the nonquantitative nature of acyl compositional analysis by CID-TOF on biological mixtures (Table 1) stems from the different propensities of acyl chains of different lipids, and in different positions on the same lipid, to produce acyl

anions. This is true at any collision offset voltage. Therefore, it is not possible to “correct” CID-TOF results by assignment of response factors to fatty acyl species, because, as shown here, response varies depending on the class and positional distribution of the fatty acid in the mixture.

Acyl species containing heteroatoms can be detected and resolved

An *Arabidopsis* leaf extract contains a mixture of the major plant phospholipids, PC, PE, phosphatidylglycerol, and phosphatidylinositol, the major plant galactolipids, MGDG, digalactosyldiacylglycerol, and sulfoquinovosyldiacylglycerol, and lesser amounts of other complex lipids (21, 22). The fatty acyl composition of *Arabidopsis* leaves differs depending on the physiological state of the plant. For example, mechanical wounding of plant leaves has been demonstrated to result in the production of a variety of oxygenated fatty acyl species, collectively known as “oxylipins” (5, 6, 8). Oxylipins can occur both as free fatty acids or esterified to the complex polar lipids of the plant plastid (6, 8, 23–27). CID-TOF MS of an unfractionated extract of *Arabidopsis* leaves subjected to wounding shows that oxylipin species can be detected (Fig. 3, Table 3), along with the normal chain acyl species. Although these same acyl species should be discernible, quantifiable, and resolvable by GC-MS, especially if a TOF analyzer is used, the advantages of CID-TOF MS for this analysis are its rapidity and the lack of requirement for derivatization or heating, which in some cases may lead to decomposition.

CID-TOF MS produces precise results

To determine the precision of the analysis, 11 samples were prepared for CID-TOF MS from the same lipid extract. The unfractionated extract was derived from the *act1* mutant of *Arabidopsis*, which has a mutation in an acyl-transferase that results in defective formation of plastidic lipids (28, 29). Thus, the *act1* mutant produces only low levels of 16:2 and 16:3. Collision energy was set at 35 eV because of the higher total signal obtained at this offset voltage compared with 60 eV. Eleven CID-TOF MS scans

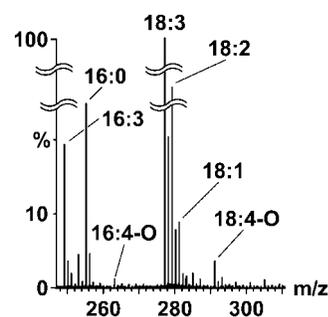


Fig. 3. CID-TOF analysis of wounded *Arabidopsis*. Centroided CID-TOF spectrum of *Arabidopsis* (Columbia ecotype) lipids at 35 eV, summary of 11 scans at 5 s/scan, after extraction from mechanically wounded leaves. Spectral data show well-defined peaks for dinoroxophytodieneoic (16:4-O; *m/z* 263) and oxophytodieneoic (18:4-O; *m/z* 291) species in the extract (see Table 3 for details). The 18:3 acyl anion (theoretical *m/z* 277.2168) was used as lock mass.

TABLE 3. CID-TOF analysis of wounded *Arabidopsis*

<i>m/z</i>	Target Chemical Formula	Interpretation	Amount Detected in Wounded <i>Arabidopsis</i>
			<i>mol%</i> of total
249.186	C ₁₆ H ₂₅ O ₂	16:3	9.41
251.201	C ₁₆ H ₂₇ O ₂	16:2	0.88
253.217	C ₁₆ H ₂₉ O ₂	16:1	1.80
255.232	C ₁₆ H ₃₁ O ₂	16:0	12.11
263.165	C ₁₆ H ₂₃ O ₃	16:4-O	0.50
277.217	C ₁₈ H ₂₉ O ₂	18:3	49.12
279.230	C ₁₈ H ₃₁ O ₂	18:2	18.64
281.248	C ₁₈ H ₃₃ O ₂	18:1	4.28
283.264	C ₁₈ H ₃₅ O ₂	18:0	0.69
289.180	C ₁₈ H ₂₅ O ₃	18:5-O	0.08
291.196	C ₁₈ H ₂₇ O ₃	18:4-O	1.68
305.248	C ₂₀ H ₃₃ O ₂	20:3	0.06
309.207	C ₁₈ H ₂₉ O ₄	18:3-2O	0.19
309.279	C ₂₀ H ₃₇ O ₂	20:1	0.06

A lipid extract of mechanically wounded *Arabidopsis* wild-type leaves was subjected to CID-TOF analysis, and increased levels of oxylipins associated with wounding were readily detected. Signals detected within an *m/z* range of ± 0.004 of the theoretical target values were assigned the interpretations shown, accounting for 99.5% of all acyl groups detected by this method.

were collected from each sample, and the results were analyzed. The data are presented in **Table 4**. The species shown account for >98% of the acyl species detected. Except for species present in very low amounts (<0.5 mol%; e.g., 16:2), the precision of the analyses was excellent (Table 4).

The simplicity and rapidity of CID-TOF MS make it a useful tool for screening biological samples

CID-TOF MS can be used to screen biological samples for alterations in fatty acid composition. For example, mutations that affect fatty acid desaturation in *Arabidopsis*

TABLE 4. Analytical precision of repetitive CID-TOF experiments

Acyl Group	Average Composition	Standard Deviation	Relative Deviation	Maximum Value	Minimum Value	Range
	<i>mol%</i>		%	<i>mol%</i>	<i>mol%</i>	
16:3	0.54	0.02	2.9	0.57	0.51	0.06
16:2	0.31	0.10	33.3	0.36	0.00	0.36
16:1	1.59	0.06	3.8	1.71	1.51	0.20
16:0	8.54	0.15	1.7	8.78	8.33	0.45
18:3	57.74	0.79	1.4	59.11	56.60	2.51
18:2	22.36	0.55	2.5	23.00	21.50	1.50
18:1	6.23	0.13	2.1	6.36	5.96	0.40
18:0	0.73	0.02	2.3	0.74	0.69	0.05

Analytical precision was evaluated by conducting independent CID-TOF analyses upon 11 aliquots of the same lipid extract. The extract was derived from the *act1* mutant of *Arabidopsis*, which has a defect in the formation of plastidic lipids and produces only low levels of 16:2 and 16:3. Summary scans representing 55 s of acquisition time were used for the analysis (11 scans of 5 s each). The tabulated acyl groups represented >98% of the combined intensity of all target acyl anions detected. Except for species present in very low amounts (<0.5 mol%; i.e., 16:2), measurements were repeatable to within a 4% relative deviation. Positive identification of an acyl anion in any given trial required that the measured (centroided) *m/z* value fall within ± 0.004 of the theoretical *m/z* value. This caused the 16:2 acyl anion to remain undetected in 1 of the 11 trials. For the 10 trials in which the 16:2 anion was positively identified, the relative deviation for the mean intensity determination was only 2.6%.

are easily discernible by CID-TOF MS. **Figure 4** shows mass spectra of unfractionated extracts from wild-type *Arabidopsis* (Fig. 4A) and three fatty acid desaturase (*fad*) mutants (Fig. 4B–D). Clearly, the *fad2* mutant shows much higher levels of the 18:1 acyl chain than the wild type as a result of its inability to convert 18:1 to 18:2. Likewise, *fad3* shows an increased level of 18:2 and *fad7* shows increased levels of 18:2 and 16:2 compared with levels in wild-type *Arabidopsis*. The ability of CID-TOF MS to detect and resolve normal chain fatty acyl species and acyl species containing heteroatoms makes this technique suitable for rapid screening of biological samples to identify both genetic alterations and physiological conditions that result in acyl species alterations. It is anticipated that once samples are identified by CID-TOF MS screening as being different from control or wild-type samples, a more detailed structural analysis of these samples will be undertaken by conventional GC and/or MS methods.

In conclusion, CID-TOF MS methodology provides a rapid screening tool enabling the detection and accurate identification of numerous acyl species from unpurified biological extracts. Time-consuming derivatization, puri-

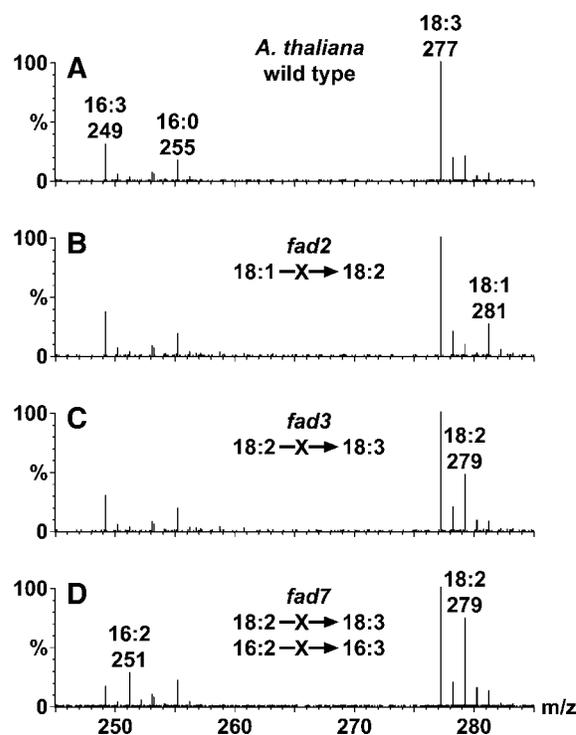


Fig. 4. CID-TOF analysis of *Arabidopsis* fatty acid desaturase (*fad*) mutants. Centroided 35 eV CID-TOF summary spectra of *Arabidopsis* extracts from the wild type and three *fad* mutants, each spectrum is the sum of 11 scans at 5 s/scan. Spectrum A: Wild-type strain showing 16:3, 16:0, and 18:3 as the predominant acyl groups. Spectrum B: *fad2* mutation, in which 18:1-to-18:2 conversion is defective, leading to the accumulation of 18:1. Spectrum C: *fad3*, in which 18:2-to-18:3 conversion is defective, leading to the accumulation of 18:2. Spectrum D: *fad7*, in which both 18:2-to-18:3 and 16:2-to-16:3 conversions are defective, causing the accumulation of 18:2 and 16:2 acyl chains. The 18:3 acyl anion was chosen as a convenient high-abundance lock mass (*m/z* 277.2168).

fication, and analysis steps are not required. Although not absolutely quantitative, the resultant data allow quick detection of samples with alterations in fatty acid composition, which may enable the elucidation of correlated genetic or physiologic alterations. 

NOTE ADDED IN PROOF

Strictly speaking, the TOF analyzer is not a scanning device. We have used the term “scans” in connection with TOF spectra loosely in place of the more cumbersome term “duty cycle.”

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REFERENCES

- Kesselmeier, J., and E. Heinz. 1987. Separation of molecular species of plant glycolipids and phospholipids by high-performance liquid chromatography. *Methods Enzymol.* **148**: 650–661.
- Christie, W. W. 1982. *Lipid Analysis*. 2nd edition. Pergamon Press, Oxford, England. 73–79.
- Mueller, M. J., L. Mène-Saffrané, C. Grun, K. Karg, and E. E. Farmer. 2006. Techniques for molecular analysis. Oxylipin analysis methods. *Plant J.* **45**: 472–489.
- Schulze, B., R. Lauchli, M. M. Sonwa, A. Schmidt, and W. Boland. 2006. Profiling of structurally labile oxylipins in plants by in situ derivatization with pentafluorobenzyl hydroxylamine. *Anal. Biochem.* **348**: 269–283.
- Vollenweider, S., H. Weber, S. Stolz, A. Chételat, and E. E. Farmer. 2000. Fatty acid ketodienes and fatty acid ketotrienes: Michael addition acceptors that accumulate in wounded and diseased *Arabidopsis* leaves. *Plant J.* **24**: 467–476.
- Weber, H., B. A. Vick, and E. E. Farmer. 1997. Dinor-oxophytodienoic acid: a new hexadecanoid signal in the jasmonate family. *Proc. Natl. Acad. Sci. USA.* **94**: 10473–10478.
- Hamberg, M., A. Sanz, and C. Castresana. 1999. α -Oxidation of fatty acids in higher plants. Identification of a pathogen-inducible oxygenase (piox) as an α -dioxygenase and biosynthesis of 2-hydroperoxylinolenic acid. *J. Biol. Chem.* **274**: 24503–24513.
- Buseman, C. M., P. Tamura, A. A. Sparks, E. J. Baughman, S. Maatta, J. Zhao, M. R. Roth, S. W. Esch, J. Shah, T. D. Williams, et al. 2006. Wounding stimulates the accumulation of glycerolipids

- containing oxophytodienoic acid and dinor-oxophytodienoic acid in *Arabidopsis* leaves. *Plant Physiol.* **142**: 28–39.
- Heinz, E. 1971. Semisynthetic galactolipids of plant origin. *Biochim. Biophys. Acta.* **231**: 537–544.
- Laudert, D., and E. W. Weiler. 1998. Allene oxide synthase: a major control point in *Arabidopsis thaliana* octadecanoid signalling. *Plant J.* **15**: 675–684.
- Welti, R., W. Li, M. Li, Y. Sang, H. Biesiada, H. E. Zhou, C. B. Rajashekar, T. D. Williams, and X. Wang. 2002. Profiling membrane lipids in plant stress responses. Role of phospholipase D α in freezing-induced lipid changes in *Arabidopsis*. *J. Biol. Chem.* **277**: 31994–32002.
- Savitzky, A., and M. J. E. Golay. 1964. Smoothing and differentiation of data by simplified least squares procedures. *Anal. Chem.* **36**: 1627–1639.
- Morris, H. R., T. Paxton, A. Dell, J. Langhorne, M. Berg, R. S. Bordoli, J. Hoyes, and R. H. Bateman. 1996. High sensitivity collisionally-activated decomposition tandem mass spectrometry on a novel quadrupole/orthogonal-acceleration time-of-flight mass spectrometer. *Rapid Commun. Mass Spectrom.* **10**: 889–896.
- Bristow, A. W. 2006. Accurate mass measurement for the determination of elemental formula—a tutorial. *Mass Spectrom. Rev.* **25**: 99–111.
- Christie, W. W. 1991. Gas chromatographic analysis of fatty acid methyl ester with high precision. *Lipid Technology.* **3**: 97–98.
- Dodds, E. D., M. R. McCoy, L. D. Rea, and J. M. Kennish. 2005. Gas chromatographic quantification of fatty acid methyl esters: flame ionization detection vs. electron impact mass spectrometry. *Lipids.* **40**: 419–428.
- Murphy, R. C. 1993. Mass spectrometry of lipids. In *Handbook of Lipid Research*. Vol. 7. Plenum Press, New York. 223–226.
- Guella, G., R. Frassanito, and I. Mancini. 2003. A new solution for an old problem: the regiochemical distribution of the acyl chains in galactolipids can be established by electrospray ionization tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* **17**: 1982–1994.
- Welti, R., W. Li, M. Li, Y. Sang, H. Biesiada, H. Zhou, C. B. Rajashekar, T. D. Williams, and X. Wang. 2002. Profiling membrane lipids in plant stress responses. Role of phospholipase D α in freezing-induced lipid changes in *Arabidopsis*. *J. Biol. Chem.* **277**: 31994–32002.
- Han, X., and R. W. Gross. 2003. Global analyses of cellular lipidomes directly from crude extracts of biological samples by ESI mass spectrometry: a bridge to lipidomics. *J. Lipid Res.* **44**: 1071–1079.
- Zien, C. A., C. Wang, X. Wang, and R. Welti. 2001. In vivo substrates and the contribution of the common phospholipase D, PLD α , to wound-induced metabolism of lipids in *Arabidopsis*. *Biochim. Biophys. Acta.* **1530**: 236–248.
- Devaiah, S. P., M. R. Roth, E. Baughman, M. Li, P. Tamura, R. Jeannotte, R. Welti, and X. Wang. 2006. Quantitative profiling of polar glycerolipid species from organs of wild-type *Arabidopsis* and a *PHOSPHOLIPASE D α 1* knockout mutant. *Phytochemistry.* **67**: 1907–1924.
- Stelmach, B. A., A. Müller, P. Hennig, S. Gebhardt, M. Schubert-Zsilavec, and E. W. Weiler. 2001. A novel class of oxylipins, *sn1-O*-(12-oxophytodienoyl)-*sn2-O*-(hexadecatrienoyl)-monogalactosyl diglyceride, from *Arabidopsis thaliana*. *J. Biol. Chem.* **276**: 12832–12838. [Erratum. 2001. *J. Biol. Chem.* **276**: 28628.]
- Hisamatsu, Y., N. Goto, K. Hasegawa, and H. Shigemori. 2003. Arabidopsides A and B, two new oxylipins from *Arabidopsis thaliana*. *Tetrahedron Lett.* **44**: 5553–5556.
- Hisamatsu, Y., N. Goto, M. Sekiguchi, K. Hasegawa, and H. Shigemori. 2005. Oxylipins arabidopsides C and D from *Arabidopsis thaliana*. *J. Nat. Prod.* **68**: 600–603.
- Creelman, R. A., and J. E. Mullet. 1997. Biosynthesis and action of jasmonates in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **48**: 355–381.
- Shah, J. 2005. Lipids, lipases, and lipid-modifying enzymes in plant disease resistance. *Annu. Rev. Phytopathol.* **43**: 229–260.
- Kunst, L., J. Browse, and C. Somerville. 1988. Altered regulation of lipid biosynthesis in a mutant of *Arabidopsis* deficient in chloroplast glycerol-3-phosphate acyltransferase activity. *Proc. Natl. Acad. Sci. USA.* **85**: 4143–4147.
- Kunst, L., J. Browse, and C. Somerville. 1989. Altered chloroplast structure and function in a mutant of *Arabidopsis* deficient in plastid glycerol-3-phosphate acyltransferase activity. *Plant Physiol.* **90**: 846–853.