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Quantitative analysis of short-chain acyl-coenzymeAs in plant tissues by LC–MS–MS electrospray ionization method

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ABSTRACT

Because acyl-CoAs play major roles in numerous anabolic and catabolic pathways, the quantitative determination of these metabolites in biological tissues is paramount to understanding the regulation of these metabolic processes. Here, we report a method for the analysis of a collection of short-chain acyl-CoAs (<6 carbon chain length) from plant extracts. Identification of each individual acyl-CoA was conducted by monitoring specific mass-fragmentation ions that are derived from common chemical moieties of all Coenzyme A (CoA) derivatives, namely the adenosine triphosphate nucleotide, pantothenate and acylated cysteamine. This method is robust and quick, enabling the quantitative analysis of up to 12 different acyl-CoAs in plant metabolite extracts with minimal post-extraction processing, using a 30 min chromatographic run-time.

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1. Introduction

Coenzyme A (CoA) is composed of three chemical moieties, an adenosine triphosphate nucleotide, pantothenate and cysteamine. The metabolically important portion of the molecule is the sulfur atom associated with the cysteamine moiety, which reacts with a carboxylic acid, to form an acyl-thioester derivative. Such acyl-CoA derivatives act as carriers of the activated acyl group, and can undergo further metabolic conversions. These acyl-CoA derivatives are critical intermediates in a number of metabolic processes, including the metabolism of fatty acids, amino acids, the initial reactions of the mevalonate pathway of isoprenoid biosynthesis, and the tricarboxylic acid cycle. Examples of acyl-CoAs include acetyl-CoA, an intermediate in many metabolic processes juxtaposed between biosynthetic and catabolic processes, and between primary and secondary metabolism; malonyl-CoA, an intermediate of fatty acid and polyketide biosynthesis; and 3-hydroxy-3-methylglutaryl CoA (HMG-CoA), the precursor of the mevalonate pathway of isoprenoid biosynthesis and an intermediate of leucine catabolism. In fact, over 20 acyl-CoAs are commercially available, and these are only a small subset of the acyl-CoAs that occur in metabolism.

Because of the functional imperative of acyl-CoAs in many metabolic processes, several methods have been developed to separate, detect, and quantify these molecules from biological samples. However, because of their low abundance, it has been difficult to generate a robust method that has the dynamic range to comprehensively detect and quantify CoA derivatives in biological extracts. For example, reverse phase-HPLC in conjunction with the inherent UV absorbance of the adenine moiety of the CoA molecule has been used to detect many of the acyl-CoAs [1]. However, the lack of sensitivity and specificity (due to the fact that metabolite extracts contain many adenine-containing metabolites) has been a limiting issue. Therefore, post-extraction derivatization chemistry has been developed to generate fluorescent-derivatives of acyl-CoAs, which can be quantified by HPLC separation coupled to a fluorescent detector [2]. Alternatively, gas chromatography methods have also been reported using FID [3] or mass spectrometer as the detector [4,5]; these methods also require post-extraction chemical derivatization.

HPLC coupled to mass spectrometry (LC–MS) has emerged as a powerful tool for analyzing acyl-CoAs, demonstrating high sensitivity while possessing the ability to confirm the structure of the molecule for validation as a part of detection. The feasibility of LC–MS/MS techniques to identify and quantify acyl-CoAs in biological samples has been described [6–12]. However, few authors have reported the analysis of acyl-CoAs in plant extracts [2,12–14].

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We report herein a method for quantitative analysis of a collection of short-chain, acyl-CoAs from plant tissue. This method can be utilized with any LC–MS/MS instrument capable of electrospray ionization (ESI).

2. Experimental

2.1. Materials

All solvents and chemicals used in this study were HPLC grade (Sigma–Aldrich, Molwaukee, WI), unless otherwise stated. All acyl-CoA standards were purchased from Sigma–Aldrich (Milwaukee, WI) as either sodium or lithium salts, and were dissolved in 10 mM ammonium formate, pH 4.6. Ammonium formate (LC–MS grade) was purchased from Fluka (Sigma–Aldrich, Milwaukee, WI). Tricholoroacetic acid (TCA) and diethyl ether were reagent grade (Sigma–Aldrich, Milwaukee, WI).

2.2. Plant growth conditions

Arabidopsis thaliana (ecotype Col-0) plants were grown from seeds in a growth room maintained at 24 °C, at 50 μE m^{-2} s^{-1} illumination. Initially, sterilized seeds were sown on MS solid media containing 0.1% (w/v) sucrose, in Petri dishes. After breaking seed dormancy by maintaining the Petri dishes at 4 °C for 4-day, the plates were transferred to a growth room, where they were held in a near-vertical position for 16-day. The aerial portions of the resultant young plants were harvested, flash frozen and stored in liquid nitrogen. For leaf and silique samples, 2-week-old plants were transplanted into soil and grown for an additional 4 weeks in a growth room. Siliques were collected 5–8 days after flowering, and leaves were collected at the same time from the same plants.

2.3. Extraction of plant tissue samples

Just before extraction, a 5 μl aliquot of 100 μM 2-butenyl-CoA was added to the frozen plant tissue (~200 mg) as an internal standard. Tissue was pulverized in extraction buffer using a Ten-Broek glass homogenizer. To optimize the extraction method, three different extraction procedures were tested. One of these used phosphate buffered propanol–acetic acid extraction buffer (propanol–acetic acid) [2], another procedure used cold 10% TCA followed by purification using an SPE column (TCA-SPE column) [10]. The third (TCA-ether partitioning), which proved to the most efficient, was as follows. Ten percentage (v/v) ice-cold TCA was added to the frozen, powderized plant material. Following homogenization and vigorous vortexing of the extract for 3 min, the TCA suspension was centrifuged at 10,000 × *g* for 10 min at 4 °C. The pellet was resuspended in 300 μl of 10% (v/v) ice-cold TCA by vortexing and the supernatant was recovered after centrifugation. The supernatants from the first and second extractions were combined and extracted with three successive partitioning with ice-cold diethyl ether, to remove the TCA. The aqueous extract was recovered, filtered through Iso-Disc™ 13 mm × 0.20 μm PTFE filter (Supelco, Bellefonte, PA), flash-frozen and lyophilized in a Speed Vac (Savant Instruments, Farmingdale, NY). The dried extract was dissolved in 50 μl of ice-cold 10 mM ammonium formate (pH 4.6) and 10 μl were injected into the LC–MS.

2.4. LC–MS–MS analysis

An Agilent MSD Ion trap mass spectrometer (model SL), coupled to an Agilent 1100 Series HPLC system that was also coupled to a diode array detector (Agilent Technologies, Palo Alto, CA) was used for analytical determinations. Chromatography was conducted with an Ascentis™ C18 (10 cm × 2.1 mm × 3 μm) column, equipped with a Supelguard™ Ascentis™ C18 (2 cm × 4.0 mm, 5 μm) precolumn (Supelco, Bellefonte, PA). Column was maintained at 25 °C, and the auto sampler for injecting the samples were maintained at 4 °C. Routinely, 10 μl of the extract was injected per analysis. LC mobile phases were: 90% 10 mM ammonium formate, pH 4.6, 10% methanol (solvent A), and 10% 10 mM ammonium formate, pH 4.6, 90% methanol (solvent B). The elution gradient was as follows: 0–5 min 100% solvent A; 5–12 min linear gradient to 100% solvent B; 12–30 min 100% solvent B; 30–35 min linear gradient to 100% solvent A. The flow rate was 0.2 ml/min.

The mass spectrometer was operated in positive ion mode with the source voltage set at 3500 V, skimmer at 40.0 V, and capillary exit at 153.9 V. Nebulizer pressure was set to 20.0 psi and 325 °C, with drying gas (nitrogen) flow rate at 8 l/min. Helium was used as the collision gas. The full scan data were obtained between *m*/*z* values of 700 and 1200 to identify the molecular ion of individual acyl-CoA molecules. In MS–MS mode, parent ions were isolated at a bandwidth of 4 AMU and after 2 ms, fragmentation was applied at 1 V, with an amplitude range of 30–200%, and scanned between *m*/*z* values of 200 and 1200. The collision energy was optimized for neutral loss and MRM (multiple reaction monitoring). The infusion experiments were carried out using seven segments within 2 min. Amplitude range from 0.5, 0.75, 1.0, 1.5, 1.75 and 2.0 V were applied in each segment. All the LC–MS experiments were obtained using 30,000 ions or 300 ms in ion charge control. MRM was used for the quantitative determination of acyl-CoAs. The quantification was based on monitoring of specific MS–MS fragments ion for each acyl-CoA. Isolated molecular ions and fragment ions were analyzed with Bruker software version 4.2 (www.bruker.com).

3. Results

3.1. Methods development

We developed an LC–MS–MS-based method to analyze CoA and a range of CoA derivatives, particularly focusing on the short-chain acyl derivatives ranging from acyl chain lengths from 2 to 6 carbons. Because of the low abundance of these molecules in plants and their inherent instability, methods for analyzing CoA derivatives need to be highly sensitive and sufficiently robust to ensure high recovery of these metabolites for quantification purposes. We therefore optimized three phases of the analytical procedure: (1) the protocol for extraction of acyl-CoAs from plant materials; (2) the LC method for separating each CoA derivative; and (3) the MS–MS protocol for identifying and quantifying each CoA derivative.

Optimizing separation of commercial acyl-CoA standards by LC was our first focus. Elution of the acyl-CoAs was detected with the mass spectrometer operated in positive mode, without fragmentation, to monitor the eluting ions (in full scan mode). Based upon prior studies, we initially used reverse-phase chromatography with the Ascentis™ amide C18 column (15 cm × 4.6 mm × 3 μm), but better separation and higher resolution was obtained by using the Ascentis™ C18 column (10 cm × 2.1 mm × 3 μm). Chromatography with this latter column was then optimized using a series of different elution gradient buffers consisting of a 10 mM ammonium formate/methanol mixture, adjusted to pH 2.2, 4.6, 7.0 and 9.0. Optimal base-line separation, narrow eluting peaks, and a minimum time of chromatography for separating 12 different CoA derivatives were obtained at pH 4.6. Fig. 1 illustrates the optimized separation of 12 acyl-CoAs.

To optimize the mass-spectrometry ionization and fragmentation parameters for distinguishing different acyl-CoAs, 50 nM solutions of each standard were infused into the mass spectrometer. These MS experiments were conducted in both positive and negative ion modes that produce protonated $(M+H)^+$ or deproto-

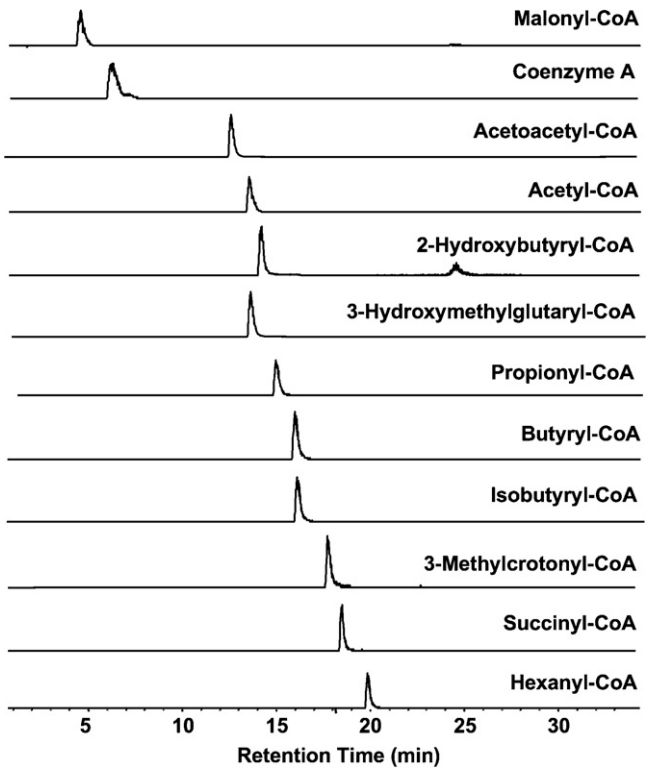

Fig. 1. Total ion chromatograms of HPLC-separated acyl-CoA standards using the chromatographic separation method described in Section 2.

nated $(M-H)^-$ ions in the chosen solvent conditions (buffered with ammonium formate at pH 4.6). The $(M+H)^+$ ions were generated more efficiently than the $(M-H)^-$ ions [15]; thus positive ion mode was found to be 3- to 5-fold more sensitive in the detection of the tested acyl-CoAs (Table 1).

MS–MS spectra were generated for the standard acyl-CoAs, so that the fragmentation patterns could be used as another criterion for identifying these molecules in biological extracts. All protonated acyl-CoAs undergo two major fragmentations, one occurs between the pantothenate moiety and the β-phosphate of the adenosine nucleotide and the second occurs between the α- and β-phosphates of the adenosine nucleotide moiety [8] (Fig. 2). The formation of an adenosine phosphate fragment plus two monophosphate fragments was observed at *m*/*z* 428 for all acyl-CoAs including free CoA [2,11,16] (Fig. 2). Loss of H_2O from this *m*/*z* 428 fragment produced another common fragment at *m*/*z* 410 (Fig. 2). In the fragmentation between the pantothenate moiety and the β-phosphate of the adenosine nucleotide, the charge associated with the $(M+H)^+$ parent ion is retained on the $(M-507+H)^+$ fragment ion, which contains the acyl-thioester-cystamine-pantothenic acid moiety (Fig. 2) [8]. As indicated in Fig. 2, the *m*/*z* value of this ion is characteristic for each acyl-CoA, and can therefore be used to identify the acyl moiety of each CoA derivative. For example, this ion has an *m*/*z* value of 303 for acetyl-CoA, 361 for 3-methylcrotonyl-CoA, and 343 for succinyl-CoA (Fig. 2, Table 1). It should be noted that this LC–MS–MS protocol does not distinguish between isomers of acyl-CoAs with identical molecular weights. For example, it is not possible to distinguish between n-butyryl-CoA and isobutyryl-CoA, based solely on this MS–MS fragmentation pattern, as these isomers generate the identical $(M-507+H)^+$ fragment ion.

The collision energy used to fragment each acyl-CoA standard was then optimized for each CoA derivative. This is illustrated with the example of the MS–MS spectra generated with propionyl-CoA using stepwise increase in the voltage of fragmentation from 0.5 to 2.0 V (Fig. 3). Based upon these data and similar results obtained with all the standard acyl-CoAs, we selected 1.0 V as the optimal collision energy to generate sufficiently abundant fragmentation ions to enable identification of each acyl-CoA.

We used MRM to identify and quantify each acyl-CoA derivative. Specifically, the abundance of three MS–MS fragment ions, M-507, 428 and 410 was quantified using MRM. These ions were selected because 428 and 410 fragments are common to all CoA derivatives, and the fragmentation reaction $(M+H)^+ \rightarrow (M-507)^+$ generates an ion that is characteristic for each acyl-CoA (Table 1). Calibration curves were generated for each acyl-CoA standard (Fig. 4). These analyses indicated that the response of the mass-detector was linear over a range of 4-orders of magnitude (from 0.1 pmol to 2 nmol). However, the response of the detector was different for each acyl-CoA, with the method being most sensitive for short-chain monocarboxylate acyl-CoAs (i.e., acetyl-CoA and propionyl-CoA), and least sensitive with dicarboxylate acyl-CoAs (i.e., malonyl-CoA and succinyl-CoA) (Table 1, Fig. 4).

Extraction of CoA derivatives from plant tissues was initially optimized by spiking extracts with a standard (2-butenyl-CoA) that is not present in the tissue. This standard was added to the frozen plant sample immediately prior to extraction. Three different extraction methods were used, varying primarily in the composition of the extraction buffer (propanol–acetic acid; TCA-SPE column; and TCA-ether partitioning, described in detail in Section 2). Based on the recovery of the 2-butenyl-CoA, the best method for optimum extraction was TCA-ether partitioning, which provided 62 (±4)% recovery (average of four determinations).

In addition, we ascertained the efficiency of extraction of individual acyl-CoAs by comparing the recovery of each acyl-CoA with and without spiking known quantities of that acyl-CoA standard to the frozen plant sample. These experiments indicated that in these optimized conditions, recovery for each acyl-CoA ranged between 20% and nearly 100% (Table 1).

Table 1
Analytical characteristics of individual acyl-CoAs.

	Extraction efficiency (%)	Retention time (min)	Ratio of detector response at positive/negative modes	Parent ion $[M+H]^+$	Characteristic fragment ion $[M-507]^+$	Detector response (peak area/pmol)
Malonyl-CoA	<20	4	4.7	854.6	347.4	10,293
Coenzyme A		6	5.6	769.1	262.1	13,321
Acetoacetyl-CoA	65	13	4.6	852.6	345.3	41,265
2-Butenyl-CoA	62	13	Not determined	836.8	329.2	76,556
Acetyl-CoA	62	14	3.7	810.7	303.2	95,683
3-Hydroxymethylglutaryl-CoA	60	14	3.9	912.3	405.5	28,927
2-Hydroxybutyryl-CoA	60	14.3	Not determined	854.6	347.2	57,846
Propionyl-CoA	62	15	3.9	824.8	317.2	90,284
Butyryl-CoA	60	16	3.1	838.8	331.2	77,812
3-Methylcrotoyl-CoA	92	18	4.5	850.7	343.2	12,707
Succinyl-CoA	100	18.5	Not determined	868.6	361.3	10,012

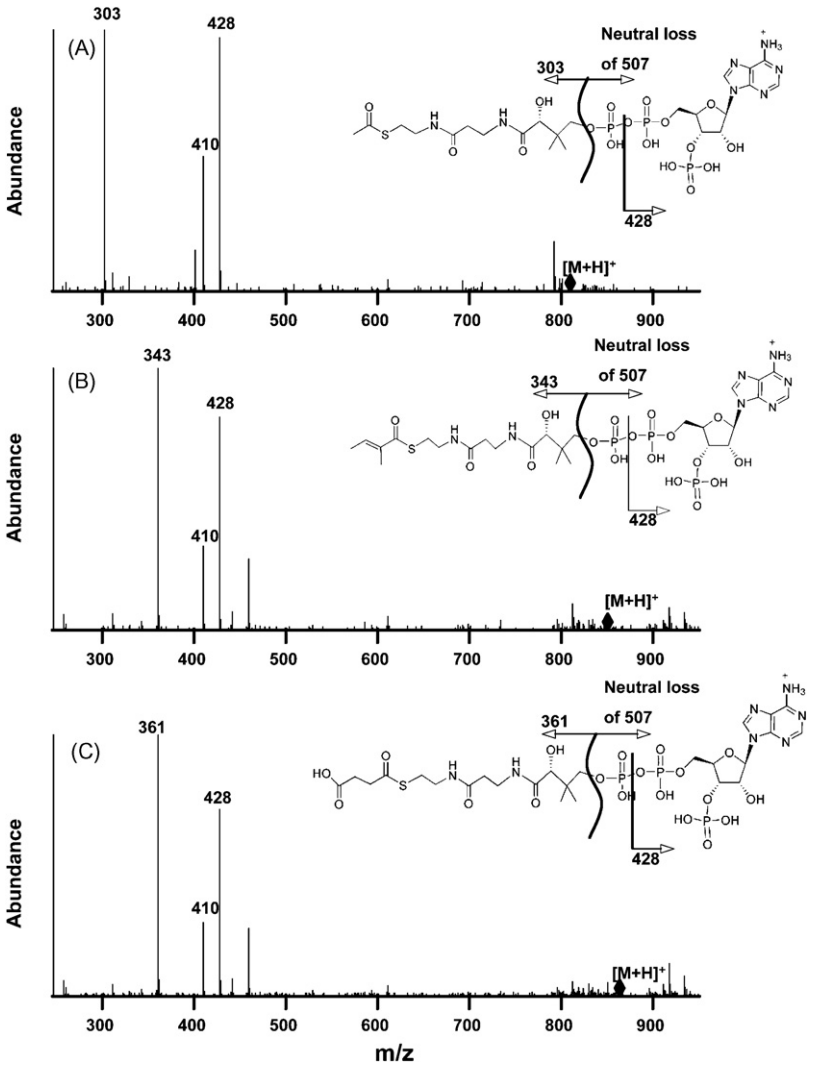

Fig. 2. MS–MS spectra of acetyl-CoA (A), 3-methylcrotonyl-CoA (B), and succinyl-CoA (C) obtained at collision energy of 1 V.

3.2. Analysis of acyl-CoAs in plant tissues

Using the optimized protocols as described in Section 3.1, we evaluated the levels of individual acyl-CoAs in plant metabolite extracts using two experimental systems. One of these had an environmental variable, comparing the levels of acyl-CoA metabolites in the aerial organs of Arabidopsis seedlings maintained either in continuous illumination during growth, or after a 3-h period when illumination was withdrawn (Fig. 5). The second experimental system, which explored changes in acyl-CoAs at two different developmental stages, compared the levels of acyl-CoAs in leaves and siliques of 6-week-old Arabidopsis plants (Table 1).

These analyses indicate that succinyl-CoA, acetyl-CoA and butyryl/isobutyryl-CoA are the most abundant acyl-CoAs in plant vegetative and reproductive organs (Fig. 5, Table 1). This finding is consistent with the fact that these metabolites are intermediates of high-flux metabolic processes, including the TCA cycle (succinyl-CoA and acetyl-CoA), fatty acid metabolism (acetyl-CoA) and amino acid metabolism (butyryl/isobutyryl-CoA), and thus might be expected to accumulate to relatively higher levels.

In contrast to the effect of changing illumination status of the plant, development had a larger impact on the abundance of these metabolites. Specifically, the acyl-CoAs accumulated to much higher levels in developing siliques than in leaves (between 2- and 25-fold difference; Table 2). The explanation for this may be multiple, but is likely due to the metabolic status of the respective organs. Namely, unlike the leaves, which were relatively mature and had ceased expansion, the siliques sampled for these analyses were undergoing rapid expansion, and the enclosed developing seeds are actively synthesizing and depositing seed reserves (i.e., oil and protein). This would imply high rates of flux in the metabolic processes that generate these seed reserves, and most of the acyl-CoAs we assayed are intermediates in these processes, therefore, their abundance might be expected to be elevated in this organ.

Two of the acyl-CoAs that we assayed, malonyl-CoA and acetoacetyl-CoA, were not consistently detected in all of the experiments. Out of 36 attempts to determine the abundance of these molecules, malonyl-CoA was detectable in only 15 independent experiments, and acetoacetyl-CoA in 26 of the 36 experiments; in those experiments where these metabolites were detected, the peak areas were near the detection limits for both of these acyl-CoAs (10 and 1 pmol, respectively). In the case of acetoacetyl-CoA, the number of experiments in which this metabolite was undetectable is equally distributed among the four different biological samples that were assayed (i.e., siliques, leaves, and seedlings maintained under either constant illumination or transiently deprived of illumination). These data may indicate therefore that the acetoacetyl-CoA concentration is near the detection limit of this method, which

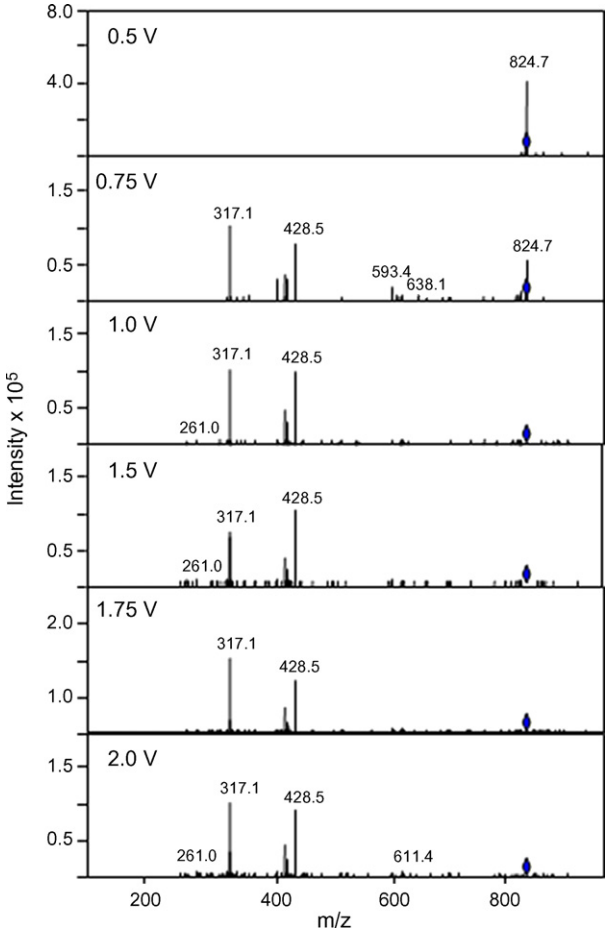

Fig. 3. MS–MS spectra of propionyl-CoA obtained at different collision energies, from 0.5 to 2.0 V. The position of the parent ion $[M+H]^+$ is marked (♦).

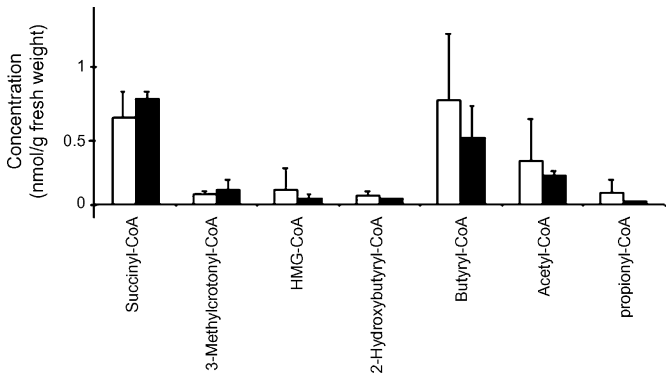

Fig. 5. Concentration of acyl-CoAs in 16-day-old Arabidopsis seedlings grown either in continuous illumination (□) or grown in continuous illumination and transferred to darkness for 3 h (■) prior to harvesting and extraction. Average of 3 analyses, ±standard deviation. *T*-Tests indicate that there is no significant difference ($P > 0.05$) in each acyl-CoA concentration upon the change of illumination status of the seedlings.

translates to approximately 0.15 nmol/g of tissue, and this concentration appears not to be substantially affected by changes in the illumination status of the plant or between leaves and siliques.

In contrast, malonyl-CoA was below the detection limit and thus undetectable in 7 out of 9 determinations for seedlings deprived of illumination, and in 8 out of 9 determinations for leaves, whereas it was near the detection limit in 6 out of 9 experiments for siliques and illuminated seedlings. These findings may therefore indicate that malonyl-CoA abundance is higher in illuminated tissues (in contrast to tissues deprived of illumination) and in reproductive organs (siliques) relative to leaves. Hence, in the tissues and treatments where malonyl-CoA was detected in some of the individual experiments, the concentration of this metabolite may be near or below the detection limit of 1.6 nmol/g of tissue.

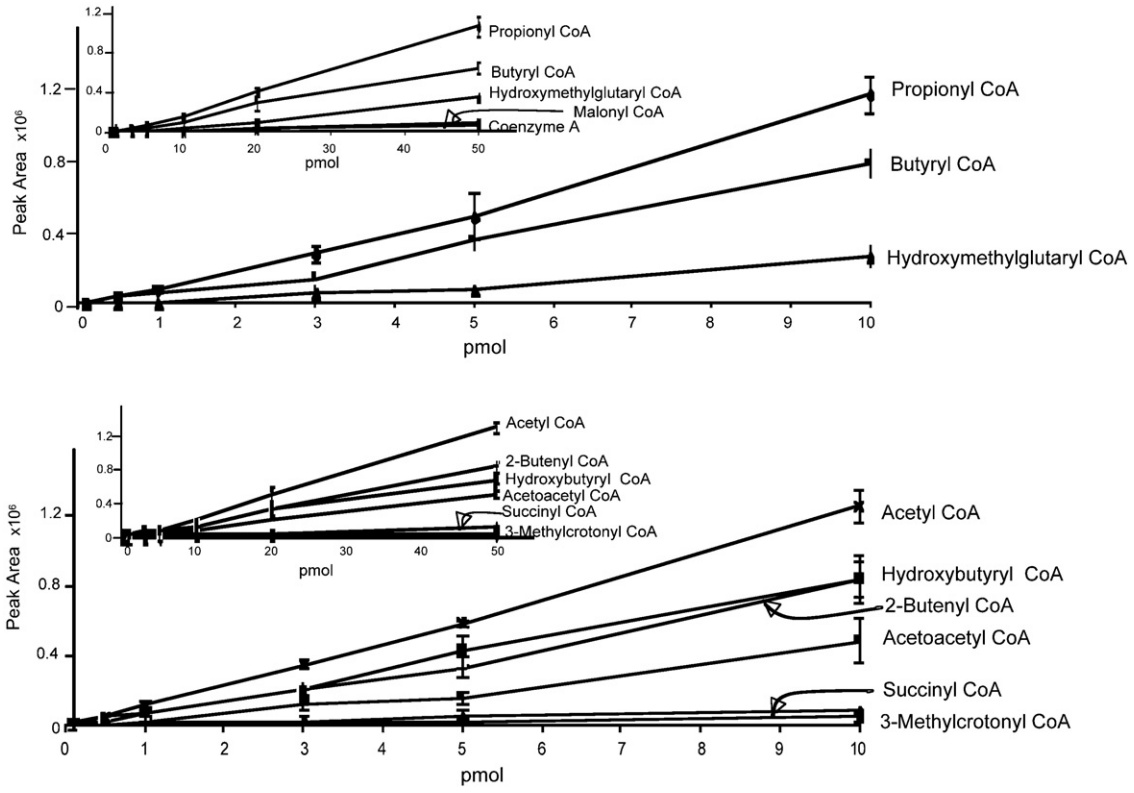

Fig. 4. Standard curves for the response of the MS-detector for each acyl-CoA.

Table 2
Acyl-CoA abundances in Arabidopsis leaves and siliques[a].

Acyl-CoA	Siliques (nmol/g fresh weight)	Leaves (nmol/g fresh weight)
Acetyl-CoA[b]	3.1 ± 2.3	0.17 ± 0.03
Propionyl-CoA	0.054 ± 0.003	0.005 ± 0.002
Butyryl-CoA/Isobutyryl-CoA	0.38 ± 0.15	0.16 ± 0.03
2-Hydroxybutyryl-CoA[b]	0.15 ± 0.10	0.017 ± 0.008
3-Methylcrotonyl-CoA[b]	0.025 ± 0.016	0.032 ± 0.023
3-Hydroxymethylglutaryl-CoA	1.1 ± 0.6	0.07 ± 0.02
Succinyl-CoA[b]	5.0 ± 0.5	0.22 ± 0.06

[a] Leaves and siliques are from 6-week-old plants, and siliques were collected 5–8 days after flowering. Average of 3 determinations ± standard deviation.
[b] *T*-Tests indicate that differences in concentrations are statistically significant ($P < 0.05$).

4. Discussion

We report herein the application of an LC–MS–MS system for the detection and quantification of short-chain acyl-CoAs in plant extracts. These molecules are unstable, difficult to detect, but are important because they participate in many metabolic processes. Therefore, the ability to quantify these metabolic intermediates should provide a means of assessing regulatory mechanisms that control different metabolic pathways. Although methods for determining the abundance of these metabolites are being increasingly reported, few have been applied to plant samples. The method that we have developed is designed for short-chain acyl-CoAs, and we have specifically optimized the extraction, the HPLC separation and the ionization parameters and MS-detection for each acyl-CoA molecule. Due to the alkali-labile nature of the thioester bond inherent in all acyl-CoAs, protocols were conducted under acidic conditions; for example, extraction was with TCA, HPLC separation was with solvents buffered with ammonium formate at pH 4.6. Ammonium formate (pH 4.6) was also used as the ionization reagent, facilitating the detection of the analytes in the positive mode.

Most prior studies of acyl-CoAs in plants have focused on determining the abundance of the fatty acid precursors acetyl-CoA, malonyl-CoA and/or, long-chain acyl-CoAs [2,4,12–14,17,18]. The experiments we report herein determined the concentrations of a broader collection of short-chain acyl-CoAs than these earlier studies, and for the first time we report the abundances of additional CoA esters, such as propionyl-CoA, butyryl-CoA/isobutyryl-CoA, 2-hydroxybutyryl-CoA, methylcrotonyl-CoA, and succinyl-CoA. Succinyl-CoA is the most abundant of the short-chain acyl CoAs whose concentrations were determined. This may not be unexpected given the fact that succinyl-CoA is an intermediate of the TCA cycle, a metabolic process that is of major significance in all aerobic organisms. However, it was somewhat surprising to find that succinyl-CoA abundance was nearly 20-fold higher in developing siliques than in leaves. This may reflect the fact that developing fruits (such as siliques) support a higher respiratory flux [19], and may thus be expected to demonstrate higher concentrations of intermediates of the TCA cycle.

Another novel finding of this study, which is consistent with expectations of the metabolic status of the tissues under study, is the finding that methylcrotonyl-CoA is more abundant in leaves than siliques. This probably reflects the fact that leucine catabolism occurs at a higher rate in leaves that siliques; methylcrotonyl-CoA being an intermediate of this mitochondrial catabolic pathway [20,21].

Prior studies attempted to evaluate the relative abundances of acetyl-CoA and malonyl-CoA, because of the importance of these two metabolites in fatty acid biosynthesis [8,13,18,22]. These studies have reported that acetyl-CoA in plants and animals is between 2 and 80 nmol/g of tissue, and that malonyl-CoA concentrations are considerably below that. We have validated our method by determining the concentrations of acetyl-CoA and malonyl-CoA in extracts, and obtained results that are consistent with these prior experiments. Acetyl-CoA levels are between 0.2 and 3 nmol/g of tissue, and malonyl-CoA levels are near or below the detection limit of our method (<1.6 nmol/g tissue).

However, it should be noted that acetyl-CoA is a common intermediate in multiple metabolic pathways (e.g., fatty acid biosynthesis, TCA cycle, fatty acid oxidation, cytosolic isoprenoid biosynthesis, flavonoid biosynthesis, fatty acid elongation; which occur in different cellular and subcellular compartments. Not all these pathways utilize malonyl-CoA as an intermediate [23], thus, a direct comparison of total acetyl-CoA and malonyl-CoA levels in tissues may not be a valid basis for making conclusions concerning the potential regulatory role of these molecules in fatty acid biosynthesis.

In summary, this manuscript reports a relatively simple LC–MS–MS method for identifying and quantifying different acyl-CoA metabolites in plant extracts. The method has wide application in many metabolic studies and can be further expanded to determine the concentrations of other acyl-CoAs, as long as chemically defined standards are available for method development and validation purposes.

Acknowledgments

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References

[1] J. Deutsch, E. Grange, S.I. Rapoport, A.D. Purdon, Anal. Biochem. 220 (1994) 312.
[2] T.R. Larson, I.A. Graham, Plant J. 25 (2001) 115.
[3] M.R. Prasad, J. Sauter, W.E.M. Lands, Anal. Biochem. 162 (1987) 202.
[4] J. Kopka, J.B. Ohlrogge, J.G. Jaworski, Anal. Biochem. 224 (1995) 51.
[5] A.E. Reszko, T. Kasumov, B. Comte, B.A. Pierce, F. David, I.R. Bederman, J. Deutsch, C. Des Rosiers, H. Brunengraber, Anal. Biochem. 298 (2001) 69.
[6] T. Mauriala, K.H. Herzig, M. Heinonen, J. Idziak, S. Auriola, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 808 (2004) 263.
[7] B. Kalderon, V. Sheena, S. Shachrur, R. Hertz, J. Bar-Tana, J. Lipid Res. 43 (2002) 1125.
[8] C. Magnes, F.M. Sinner, W. Regittnig, T.R. Pieber, Anal. Chem. 77 (2005) 2889.
[9] F. Kasuya, Y. Oti, T. Tatsuki, K. Igarashi, Anal. Biochem. 325 (2004) 196.
[10] P.E. Minkler, J. Kerner, T. Kasumov, W. Parland, C.L. Hoppel, Anal. Biochem. 352 (2006) 24.
[11] J.J. Dalluge, S. Gort, R. Hobson, O. Selifonova, F. Amore, R. Gokarn, Anal. Bioanal. Chem. 374 (2002) 835.
[12] O. Hayashi, K. Satoh, Biosci. Biotechnol. Biochem. 70 (2006) 2676.
[13] A.W. Tumaney, J.B. Ohlrogge, M. Pollard, J. Plant Physiol. 161 (2004) 485.
[14] D. Post-Beittenmiller, G. Roughan, J.B. Ohlrogge, Plant Physiol. 100 (1992) 923.
[15] C.A. Haynes, J.C. Allegood, K. Sims, E.W. Wang, M.C. Sullards, A.H. Merrill Jr., J. Lipid Res. 49 (2008) 1113.
[16] K.L. Burns, L.T. Gelbaum, M.C. Sullards, D.E. Bostwick, S.W. May, J. Biol. Chem. 280 (2005) 16550.
[17] P.G. Roughan, Biochem. J. 327 (1997) 267.
[18] Y. Gibon, H. Vigeolas, A. Tiessen, P. Geigenberger, M. Stitt, Plant J. 30 (2002) 221.

[19] H. Lambers, in: R. Douce, D.A. Day (Eds.), Higher Plant Cell Respiration, Springer–Verlag, Berlin, 1985, p. p418.
[20] M.D. Anderson, P. Che, J. Song, B.J. Nikolau, E.S. Wurtele, Plant Physiol. 118 (1998) 1127.
[21] P. Che, E.S. Wurtele, B.J. Nikolau, Plant Physiol. 129 (2002) 625.
[22] L. Gao, W. Chiou, H. Tang, X. Cheng, H.S. Camp, D.J. Burns, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 853 (2007) 303.
[23] B.L. Fatland, B.J. Nikolau, E.S. Wurtele, Plant Cell. 17 (2005) 182.