Analysis of Long-Chain Fatty Acyl Coenzyme A Thioesters by Negative Ion Fast-Atom Bombardment Mass Spectrometry and Tandem Mass Spectrometry

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Long-chain acyl Coenzyme A (CoA) is essentially composed of three major chemical groups, fatty acyl-, phosphopantetheino-, and 3',5'-adenosine diphospho-moieties. The negative ion fast-atom bombardment mass spectrometry spectra of long-chain acyl CoA thioesters were characterized by the formation of abundant $[M - H]^-$ and two distinct classes of fragment ions, one class which retained the acyl group and another class which is related to CoA that contains the phosphopantetheine and adenine. The ions which retained the acyl group in the spectrum of palmitoyl CoA appeared at m/z 675, 657, 595, and 577 and were found to decompose by loss of alkylketene observed at m/z 357 and 339. Those ions which retained the adenine group were observed at m/z 426 and 408. In contrast to these ions observed following fast-atom bombardment ionization, tandem mass spectrometry of the $[M - H]^-$, from palmitoyl CoA (m/z 1004), yielded the adenine-containing ions as major products and the acyl-containing ions were of low abundance or not detected. These results suggested that the formation of many characteristic ions observed in direct FAB analysis occurred during the desorption process. The unique relationship between ions which involved the transition from acyl-containing ions to only CoA-containing ions by the loss of alkylketene allowed the development of tandem mass spectrometry protocols for the analysis of acyl CoA mixtures. Precursor scans of either m/z 357 or 339 yielded the identification of each species in a complex mixture. Identification of specific species was obtained with a neutral loss scan of the mass for a specific alkylketene. (*J Am Soc Mass Spectrom* 1994, 5, 416-424)

Coenzyme A (CoA) thioesters of long-chain fatty acids (acyl CoA) are essential intermediates in all aspects of fatty acid synthesis [1], degradation [2], and phospholipid biosynthesis [3]. Acyl CoA thioesters are thought to play important roles in many cellular regulatory processes including biosynthesis of the lipid mediator platelet activating factor [4], and intermediates of leukotriene [5, 6] and prostaglandin metabolism [7]. In addition, acyl CoA thioesters are also formed with xenobiotics in the pathway of conjugation to endogenous amino acids [8]. Because of the atomic size of the sulfur atom in the thioester compared to the oxygen atom in esters, there is less π -electron overlap and a destabilization of the thioester bond relative to that of an ester. This makes the thiolate anion a better leaving group in nucleophilic displacement reactions that are catalyzed by various enzymes that use the acyl CoA derivative.

Long-chain acyl CoA is essentially composed of three major chemical groups: fatty acyl-, phosphopantetheino-, and 3',5'-adenosine diphospho-moieties (ADP). Several mass spectrometric strategies have been used for the analysis of acyl CoAs. Long-chain fatty alcohols formed from the reduction of acyl CoA esters have been analyzed by electron capture negative ion gas chromatography mass spectrometry as the pentafluorobenzoyl esters [9]. While this method is reported to be quite sensitive, it has the disadvantages inherent with chemical workup and derivatization. Analysis of intact acyl CoAs has involved direct and continuous-flow fast-atom bombardment mass spectrometry (FAB/MS) [10-12]. These fast-atom bombardment (FAB) studies were based upon positive ionization modes. Both $[M + H]^+$ and fragment ions indicative of the CoA and acyl group were observed, and the base peak of the spectrum was protonated adenine, m/z 136. A recent report using tandem mass spectrometry of positive ions from 3-keto-2-propylpentanoyl-CoA indicated the utility of this approach to probe detailed structural features in this

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unique CoA ester [13]. Little work has been performed to evaluate the efficacy of negative ionization of intact acyl CoAs. One previous study did report that abundant $[M - H]^-$ ions were produced from LTB₄-CoA [6]; however, this study did not describe any other ion formation. For these reasons we have investigated the negative ion FAB/MS and fast-atom bombardment tandem mass spectrometry (FAB/MS/MS) behavior of long-chain acyl CoAs.

Methods

Standard long-chain acyl CoA species, in their free acid form, were obtained from Sigma Chemical Company (St. Louis, MO). Individual and mixture of standards were dissolved in methylene chloride/methanol (2/1) to give a final concentration of 1 µg/ul per each compound. Aliquots (1 ul) were applied to the FAB probe using a variety of matrices (reagent grade) as described in the Results. $[^2\text{H}]_{10}$ -(N,O)-Palmitoyl-CoA was prepared by dissolving 10 µg of palmitoyl-CoA in 100 ul of ethanol-OD (99.5% D, Aldrich, Milwaukee, WI), evaporation of the solvent in vacuo, and repeating the procedure three times. $[^2\text{H}]_{10}$ -(N,O)-Palmitoyl-CoA was analyzed using $[^2\text{H}]_3$ -glycerol (98% D, Aldrich, Milwaukee, WI) as the FAB matrix. The most abundant isotopic species observed was $[^2\text{H}]_{10}$ -(N,O)-palmitoyl-CoA which was selected for collision-induced dissociation (CID) and analysis by tandem mass spectrometry. Other species were observed at lower abundances containing eight and nine deuterium atoms indicating incomplete deuterium exchange, but these species did not interfere with the CID experiments.

Analyses were performed on a Finnigan TSQ 70 mass spectrometer (Finnigan Corporation, San Jose, CA) equipped with a FAB gun from Ion Tech (Teddington, England). Scans were obtained over the range m/z 200 to 1200 u at the rate of 2 s/scan. The FAB gun was operated with xenon as the particle source at 6 keV. Tandem mass spectrometry employed a collision energy of 30 eV (laboratory frame of reference) with argon as the collision gas at a pressure of 0.5 mTorr.

Results

FAB / MS (Source-formed ions)

With negative ion FAB/MS desorption and ionization each group, fatty acyl-, phosphopantetheino-, and 3',5'-adenosine diphospho-moieties (Scheme I) exerted an influence to yield fragment ions characteristic of that group. Indeed, long-chain acyl CoA thioesters produced abundant $[M - H]^-$ ions and two distinct classes of fragment ions, one class retaining the acyl group (designated I-V) and the other related to coenzyme A and/or adenosine diphosphate (designated A-E). The class of ions (A-E) related to the CoA or ADP groups are common to all acyl CoAs while acyl-containing ions (I-V) shift in mass according to the mass of the acyl group. This is illustrated in Figure 1

with the negative ion spectra of palmitoyl- and arachidonyl-CoA which displayed $[M - H]^-$ at m/z 1004 and 1052, respectively. (Even though the number of hydrogen atoms in acyl CoA species is relatively high (66-74 protons), the exact masses observed for molecular ion species is only 0.2-0.4 above the nominal mass because of the number of mass deficient heteroatoms also present. The exact molecular masses for palmitoyl and arachidonyl CoA are 1005.3 and 1052.3, respectively. In this publication we will report the nominal masses for observed ions rather than the exact masses.) The major fragment ions of each class are described below. The assignment of ion structures is supported by appropriate mass shifts between the spectra of palmitoyl- and $[^2\text{H}]_{10}$ -(N,O)-palmitoyl-CoA as depicted below in specific ion structures.

Figure 1. Negative ion FAB/MS of (a) palmitoyl CoA and (b) arachidonyl CoA obtained in glycerol matrix; letter m designates matrix ions.

Formation of most negative ions can be rationalized by an initial hydrogen abstraction from and charge localization on one of the phosphate hydroxyl groups. Fragmentation of the 5'-phosphate ester (adenosine-3'-monophosphate (AMP)) bond of palmitoyl-CoA with charge retention on the acyl diphosphopantetheine group yields the ion observed at m/z 675 (*I*), while m/z 657 (*II*) nominally represents an additional loss of H_2O . In the spectrum of $[^2H]_{10}$ -(N,O)-palmitoyl-CoA these ions were shifted to m/z 680 and 660, respectively, corresponding to a loss of D_2O . This loss of water involving exclusively exchangeable protons is consistent with the formation of a cyclic phosphate ester bond.

Ion I
 m/z 675 (680)

Ion II
 m/z 657 (660)

The ions observed at m/z 595 and 426 (*III* and *B*, respectively) may be formed by fragmentation on either side of the central oxygen in the diphospho ester bridge between AMP and acyl pantetheine. Thus *III*, an acyl diagnostic ion, may be formed with charge

retention on the acylphosphopantetheine and *B*, a CoA diagnostic ion, may be formed with charge retention on the 5'-phospho-AMP.

Ion III
 m/z 595 (599)

Ion B
 m/z 426 (432)

ions *C* (m/z 408) and *IV* (m/z 577) are most likely dehydrated analogs of *B* and *III*, respectively. In the spectrum of $[^2H]_{10}$ -(N,O)-palmitoyl-CoA these ions were shifted to m/z 599 (*III*), 579 (*IV*), 432 (*B*), and 412 (*C*), again consistent with the formation of cyclic phosphate esters in *IV* and *C*.

Ion IV
 m/z 577 (579)

Ion C
 m/z 408 (412)

The ions observed at m/z 357 (*D*) and m/z 339 (*E*) in the mass spectrum of palmitoyl-CoA were also observed in the mass spectra of all other acyl CoAs studied (Figure 1 and Table 1). One reasonable pathway for the formation of these ions (*D*) and (*E*) is by loss of alkylketene from the acyl phosphopantetheine ions, *III* and *IV*. The significance of this transition is

Table 1. Abundance of source-formed fragment ions from fatty acyl CoAs

Fatty Acyl Group ^b	Abundance of CoA Diagnostic Fragment Ions from Acyl CoA ^a				
	Fragment Ion				
	A	B	C	D	E
	m/z 766	m/z 426	m/z 408	m/z 357	m/z 339
16:0	12	14	19	53	47
17:0	7	16	23	53	65
18:1	15	30	58	33	93
18:0	16	12	24	38	60
19:0	12	18	25	50	49
20:4	32	37	63	88	93
20:0	28	9	17	43	70

[M - H] ⁻	Abundance of Fatty Acyl Diagnostic Fragment Ions from Acyl CoA ^a				
	Fragment Ion ^c				
	I	II	III	IV	V
16:0	1004 (42)	675 (33)	657 (12)	595 (116)	271 (100)
17:0	1018 (23)	689 (32)	671 (12)	609 (68)	285 (100)
18:1	1030 (40)	701 (28)	683 (16)	621 (48)	297 (100)
18:0	1032 (40)	703 (38)	685 (14)	623 (52)	299 (100)
19:0	1046 (28)	717 (25)	699 (10)	637 (58)	313 (100)
20:4	1052 (54)	723 (46)	705 (24)	643 (95)	319 (100)
20:0	1060 (20)	731 (31)	713 (14)	651 (41)	327 (100)

^aSpectra were obtained in glycerol and abundances listed are normalized to ion V (100).

^bAcyl nomenclature cc:b, where cc is the carbon chain length and b is the total number of double bonds.

^cData are listed as mass-to-charge ratio values with relative abundances in parentheses.

important because the fatty acyl containing ions (III and IV) decompose to CoA diagnostic ions (D and E, respectively) which lack the fatty acyl group.

The other two ions, A and V, observed at m/z 766 and 271 in the spectrum of palmitoyl-CoA (Figure 1) correspond to fragmentation on either side of the thioester bond. Loss of alkylketene from $[M - H]^-$ ion yields A, m/z 766, an ion analogous to the $[M - H]^-$ from free CoA. Interestingly, this ion shifted by 9 u to m/z 775 in the spectrum of the deuterated analog indicating the loss of one deuterium. Scission of the sulfur bond between thioester and pantetheine yields another important acyl diagnostic ion V, the acyl thiolate anion.

A series of seven saturated and unsaturated long chain-acyl CoAs (16:0, 17:0, 18:0, 18:1, 19:0, 20:0, and 20:4) were studied under negative ion FAB/MS using a variety of matrices. The negative ion FAB mass spectra of this series of long-chain acyl CoAs are summarized in Table 1 (glycerol matrix). The most abundant fragment ions are the acyl-specific ions III, IV, and V, and the CoA diagnostic ions D and E. The least abundant fragment ions formed were the CoA related A, B, and C ions.

The matrices employed included thioglycerol, glycerol, diethanolamine, and triethanolamine. In general, spectra containing abundant $[M - H]^-$ and fragment ions, in particular abundant type V ions, were obtained with the more basic matrices, diethanolamine and triethanolamine. Spectra generated in the presence of the relatively more acidic matrix, thioglycerol, also yielded abundant $[M - H]^-$ ions and more abundant acyl-containing ions III and IV, but less abundant thiolate type anions V (Table 2).

From the perspective of identifying specific acyl-containing CoA thioesters, negative ion FAB/MS demonstrated good performance and prompted further study of their CID behavior to evaluate specific decompositions for the analysis of complex mixtures.

FAB/MS/MS:

Tandem mass spectrometry included product ion, precursor ion, and neutral loss scans following CID studies of the long-chain acyl CoAs to investigate the formation of the fragment ions observed in FAB/MS spectra. Product ions formed from CID of the major source-formed ions from palmitoyl CoA are summarized in Table 3 and listed in comparison with the FAB/MS spectrum. Product ion spectra from the other acyl CoA species were qualitatively similar.

$[M - H]^-$ (m/z 1004). As shown in Figure 2, the most abundant product ions formed from CID of the $[M - H]^-$ of palmitoyl-CoA are the CoA class ions, B and C (m/z 426 and 408, respectively). The acyl-containing ions were either of low abundance, I (m/z 675), II (m/z 657), and IV (m/z 577) or completely absent, III (m/z 595) and V (m/z 271). Also absent were the CoA ions D and E (m/z 357 and 339, respectively), which are derived from acyl-containing ions by the loss of alkylketene. The product ion spectrum is in contrast to the FAB/MS analysis in which the most abundant ions observed were acyl-containing fragments and the least abundant ions were CoA related (Figure 1a). Other minor product ions were observed at m/z 924, loss of 80 u (HPO_3) from $[M - H]^-$, and two fragments not seen in the FAB/MS spectrum, m/z 328 and 273. These two ions, m/z 328 and 273, most likely derive from ions B (m/z 426) and C (m/z 408) by losses of 98 u (H_2PO_4) and 135 u (adenine), respectively.

Ion I (m/z 675). The product ion spectrum of I from palmitoyl CoA is shown in Figure 3. Two abundant ions m/z 577 and 339 (IV and E, respectively) are formed by an initial loss of H_3PO_4 (98 u) and the loss of H_3PO_4 followed by the loss of hexadecylketene (238 u), respectively. These losses are supported by the CID of this ion (m/z 680) from $[^2H]_{10}$ -(N,O)-palmitoyl-CoA in which both product ions lost three deuterium atoms (as 2H_3PO_4) and the observed product ions, retaining

Table 2. Matrix effects on observed abundances of source-formed fragment ions from palmitoyl CoA^a

Matrix	$[M - H]^-$	A	B	C	D	E	I	II	III	IV	V
m/z	1004	766	426	408	357	339	675	657	595	577	271
TEA	27	6	14	14	12	21	12	7	34	37	100
DEA	26	7	8	10	17	12	11	5	28	31	100
Glycerol	36	10	12	16	46	40	28	10	100	91	86
Thioglycerol	25	6	20	20	47	48	16	76	77	100	46

^aIon abundances are from an averaged spectrum of five scans, normalized to the most abundant ion.

Figure 2. Product ions obtained following collision induced dissociation (30 eV collision energy, 0.5 mTorr argon) of $[M - H]^+$ ions of palmitoyl CoA.

two deuterium atoms, were shifted by 2 u to m/z 579 and 341, respectively (Table 3). Also the formation of ion *E* by loss of alkylketene was supported by the observation of this ion in the FAB/MS spectra of all other acyl CoAs studied (Table 1) as well as in their CID spectra of the respective *I* type ions (data not shown). Other products ions, *II*, *III*, *D*, and *V* (m/z 657, 595, 357, and 271, respectively) were also observed in the FAB/MS spectrum. Of these ions, *III* and *D* were minor ions in contrast to the FAB/MS analysis in which they were very abundant. Product ions not observed in the FAB/MS spectrum included m/z 305,

Figure 3. Product ions obtained following collision induced dissociation (30 eV collision energy, 0.5 mTorr argon) of m/z 675, Type *I* ions from palmitoyl CoA.

208, and 159. These product ions were unchanged in observed mass in the CID of *I* from the other long-chain acyl CoAs studied and therefore did not retain the acyl group. The ion at m/z 305 was also observed in the CID spectra of *II* (m/z 657) and *IV* (m/z 577) as listed in Table 3, and the formation of this product ion most likely involves loss of the thioacid (RCOSH, 272 u) from *IV* (m/z 577). That this ion is shifted by only 1 u in the CID spectrum of the deuterated analog

Table 3. FAB/MS and FAB/MS/MS ions from palmitoyl CoA

FAB/MS ^{a,c}	$[M - H]^+$	Collision Induced Dissociation Ion ^{a,b}			
		<i>I</i>	<i>II</i>	<i>III</i>	<i>IV</i>
1004 (1013)	1004				
924	924				
766 (775)					
675 (680)	675	675 (680)			
657 (660)	657	657 (660)	657 (660)		
595 (599)		595 (595)		595 (599)	
577 (579)	577	577 (579)	577 (579)		577 (579)
	488				
426 (432)	426				
408 (412)	408				
357 (361)		357 (361)	357 (361)	357 (361)	
	328				
339 (341)		339 (341)	339 (341)	339 (341)	339 (341)
				323 (326)	
		305 (306)	305 (306)		305 (306)
	273				
271 (271)		271 (271)	271 (271)	271 (271)	271 (271)
		208 (209)	208 (209)		208 (209)
					193
					163 (163)
		159 (160)	159 (160)		

^aMass-to-charge ratio values in parentheses are from $[^2H]_{10}$ -(N,O)-palmitoyl CoA.

^bMass-to-charge ratio values of precursor ions selected for CID are underlined.

^cSource-formed ions observed in FAB/MS.

implies a deuterium transfer from the amide nitrogen, perhaps to form an oxazole ring (Scheme II). The fragment ion at m/z 159 is consistent with $[\text{HP}_2\text{O}_6]^-$ and this ion also shifted by 1 u in the CID spectrum of the deuterated analog (Table 3). The other ion (m/z 208) is a common product ion in the CID spectra of *I*, *II*, and *IV*, and the formation of this ion most likely involves the loss of $\text{RCOSCH}_2\text{CH}_2\text{NHCOCH}=\text{CH}_2$ from m/z 577 (*IV*) perhaps via a McLafferty-like γ -proton rearrangement (Scheme III). The resultant structure of this ion retains only one exchangeable hydrogen and is in agreement with a 1 u shift to m/z 209 in the CID spectrum of the deuterated analog (Table 3).

Ion II (m/z 657). The CID spectrum of this ion from palmitoyl CoA was quite similar to that of *I*, except that the abundance of *IV*, m/z 577, was very low in this case and no type *III*, m/z 595, was observed. All other product ions listed in Table 3 are as described above in the CID spectrum of *I*, m/z 675.

Ion III (m/z 595). A single abundant ion, m/z 357

(*D*) dominated the product ion spectrum of *III* as shown in Figure 4. This is a common ion observed in CID of *III* from all other acyl CoAs and is formed by the neutral loss of alkylketene, in this case hexadecylketene (238 u). Low abundance ions observed in the spectrum included m/z 339, 323, and 271. Of these, m/z 339 (*E*) and 271 (*V*) have been discussed above. The ion at m/z 323, which is observed in the CID of *III* from all other acyl CoAs, is derived by loss of the acylthioacid (RCOSH , 272 u) as in the formation of m/z 305 in the CID spectrum of *I* (Scheme II). This ion shifted by 3 u in the CID of the deuterated analog, which again implied the transfer of a deuterium atom from an amide to form the neutral thioacid.

Ion IV (m/z 577). The CID behavior of *IV* was similar to that observed with *III* in that a single abundant product ion was formed by the loss of the neutral alkylketene, resulting in the ion at m/z 339 (*E*). Other ions observed in the spectrum (Table 3) were of very low abundance.

Ion B (m/z 426). This ion, which does not contain the acyl group, produced four ions with collisional activation. The ions observed were m/z 408 (loss of water), 328 (loss of H_2PO_4), 159 ($[\text{HP}_2\text{PO}_6]^-$), and 134 (adenine - H) (data not shown).

Ion C (m/z 408). The product ion spectrum of *C* contained only one ion not observed in decomposition of *B*. This ion, m/z 273, which was also observed in the product ion spectrum of the $[\text{M} - \text{H}]^-$, is most likely due to the loss of adenine, 135 u (data not shown).

Ion V (m/z 271). The acyl thiolate anion, $[\text{RCOS}]^-$, is unique for each acyl CoA studied and is analogous to the carboxylate anion derived from negative ion FAB/MS of fatty acids and phospholipids [14-16]. The CID of *V* did not yield many product ions. However,

Figure 4. Product ions obtained following collision induced dissociation (30 eV collision energy, 0.5 mTorr argon) of m/z 595, Type *III* ions from palmitoyl CoA.

increasing the collision offset (Q_2) potential to 100 V did produce a low abundance series of product ions differing in 14 u, beginning at m/z 60, $[\text{SCO}]^-$. The abundance of this ion series was not sufficient to determine double bond positions in unsaturated acyl CoAs, as has been demonstrated in the high energy CID of carboxylate anions from fatty acids [14-16]. Precursor ion spectra in the formation of this ion (m/z 271) contained only one abundant ion, IV, and to a much lesser extent, III (m/z 577 and 595, respectively, for palmitoyl CoA).

Analysis of Acyl CoA Mixtures by FAB/MS/MS

The product ion spectra of III and IV from all acyl CoA species studied contained abundant ions from the loss of alkylketene at m/z 357 and 339 (D and E, respectively). The precursor scans of these ions, D and E, also contained only abundant ions corresponding to III and IV, respectively (data not shown). As D and E are fragment ions common to all acyl CoA species, precursor scans of these ions during FAB/CID of mixtures of acyl CoA thioesters contained ions diagnostic of each acyl CoA species in the mixture.

Figure 5a shows the negative ion FAB/MS of a mixture of seven standard acyl CoA thioesters with the inset expanding the mass-to-charge ratio region containing the III and IV type ions from each of the CoA species present. A much simpler spectrum results from CID and precursor scans of m/z 339, E (Figure 5b). In this spectrum, only IV ions are detected and the expanded region in Figure 5b reveals ions clearly indicating each acyl CoA species present in the mixture.

An alternate strategy would be to use CID and tandem mass spectrometric analysis to search a mixture of acyl CoA esters for the presence of one or more specific molecular species. Neutral loss scans of a specific alkylketene would detect only the transition of the III and IV type ions to E (m/z 357) and D (m/z 339) ions associated with that specific molecular species. This is demonstrated in Figure 5c with the same acyl CoA mixture employing neutral loss scans of 238 u (loss of hexadecylketene), a specific loss for palmitoyl CoA. As shown only m/z 595 and 577 (III and IV, respectively from palmitoyl CoA) are observed (Figure 5c).

Discussion

The negative ion FAB/MS spectra of long-chain acyl CoA thioesters are characterized by the formation of abundant $[\text{M} - \text{H}]^-$ and two distinct classes of fragment ions, one class which retains the acyl group and another class which is related to CoA. For the identification of specific acyl CoA species the first series of fragment ions (I-V) are obviously more important as these ions retain the long-chain fatty acyl group. With

Figure 5. (a) FAB/MS of a mixture of acyl CoAs containing C16:0, C17:0, C18:1, C18:0, C19:0, C20:0, and C20:4 acyl species. The spectrum was obtained from a glycerol matrix and the letter m designates matrix ions; (b) precursor ions of m/z 339 obtained following collision induced dissociation (30 eV collision energy, 0.5 mTorr argon) of source formed ions, m/z 350-1100 from the same mixture of acyl CoA species; and (c) precursor ions of the neutral loss of hexadecylketene, 238 u, obtained following collision induced dissociation (30 eV collision energy, 0.5 mTorr argon) of source formed ions, m/z 300-700, from the same mixture of acyl CoA species.

negative ionization the class of fragment ions retaining the acyl group are much more abundant than the CoA related class of ions (Table 1).

The most probable site of the initial negative ionization is one of the three phosphate groups and many of the fragment ions are derived from fragmentation of a phospho anhydride or ester bond and subsequent losses of either water or alkylketene. As has been described above, the ions which retain the acyl group in the spectrum of palmitoyl CoA appear at m/z 675, 657, 595, and 577. Ions formed from these by loss of alkylketene are m/z 357 and 339. Fragment ions which retain the adenine group are observed at m/z 426 and 408 (Figure 1). In contrast to these ions observed following FAB ionization, tandem mass spectrometry of

the $[M - H]^-$, from palmitoyl CoA (m/z 1004), yielded the adenine-containing ions as major products and the acyl-containing ions were of low abundance or not detected. In addition, the most abundant fragment ion observed in the FAB/MS spectrum, the thiolate anion (m/z 271), was not observed at all (as well as the lesser abundant ion at m/z 766). These results suggested that the formation of many characteristic ions observed in direct FAB analysis occurred during the desorption process. Although the excitation and decomposition of source-formed ions can be quite different from collisional activation with respect to energy and time considerations, other observations argue that these are not the only parameters affecting fragmentation. The observed abundances of fragment ions in the FAB/MS spectra were dependent upon the matrix employed. With relatively basic matrices such as diethanolamine and triethanolamine, the most abundant fragment ions formed were the thiolate anions (V). In contrast, with less basic matrices such as glycerol and thioglycerol, the most abundant ions formed were the type III and IV (Table 2) which involve fragmentation of the phospho anhydride bond. These observations argue for an active role of either matrix molecules or matrix-derived anions in the formation of fragment ions rather than ionization solely at a phosphate residue. Such interactions occurring in the seldedge region during desorption and ionization of both positive and negative ions have been reviewed [17] and previously observed in the negative ion FAB/MS of another phosphate-containing lipid, glycerophosphocholine [18].

Fragmentation of the central phospho anhydride bond can form either an acylpantetheinophosphate anion or a diphosphoadenosine anion. The abundant type III ions (m/z 595 in palmitoyl CoA) observed in the FAB/MS spectrum suggest that the formation of the acylpantetheinophosphate anion was greatly favored over the formation of the diphosphoadenosine anion, m/z 426. Fragmentation on either side of the anhydride bond may be equally favored, but the strongly basic amino sites in adenine may also abstract a proton from other matrix molecules to form a neutral zwitterionic diphosphoadenosine species. This would result in the observed greater abundance of the acyl-containing species. In the absence of matrix molecules, fragmentation of this anhydride bond also occurred, as in the CID spectrum of the $[M - H]^-$ from palmitoyl CoA (Figure 2). In this decomposition mode charge retention by the diphosphoadenosine was favored. Without the presence of matrix molecules during CID, formation of the diphosphoadenosine anion may be favored with charge localized over two phosphate groups, while the acylpantetheinophosphate contains only one such group to stabilize the charge.

Positive ionization FAB/MS of acyl CoAs also yielded abundant $[M + H]^+$ and acyl retaining fragment ions [10, 11]. Two major differences were observed between positive and negative ion spectra: the

base peak observed with positive ionization was protonated adenine, m/z 136, while the base peak with negative ionization was typically the acyl thiolate anion, V. The acyl thiolate anion was not observed in the positive ion FAB spectrum of acyl CoAs, and the formation of this ion in the negative ionization mode, especially when enhanced by the presence of a basic matrix, may be particularly useful in the identification of isomeric long-chain acyl CoAs. Clearly, tandem mass spectrometric analysis of both positive and negative ions generated from long-chain fatty acyl CoA esters provide structurally useful and complementary information.

The use of CID and tandem mass spectrometric techniques with negative ion formation will undoubtedly be useful in the analysis of complex mixtures of acyl CoAs. The unique relationship between type IV and E or III and D ions which involve the transition from acyl-containing ions to only CoA-containing ions allows the development of tandem mass spectrometry protocols similar to those developed for the analysis of phospholipid mixtures. Such techniques have been used with good success with phosphocholine mixtures to determine both molecular species [19, 20] and to identify novel individual species [21]. These techniques are directly adaptable to mixtures of acyl CoAs, using either precursor or neutral loss scans.

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