Negative Ion Tandem Mass Spectrometry of Leukotriene E₄ and LTE₄ Metabolites: Identification of LTE₄ in Human Urine

Angelo Sala*, Kathleen Kayganich, Joseph A. Zirrolli, and Robert C. Murphy

National Jewish Center for Immunology and Respiratory Medicine, Denver, Colorado, USA

The sulfidopeptide leukotrienes, leukotriene E₄ (LTE₄) and its N-acetyl derivative and several ω - and β -oxidized metabolites of LTE₄, have been analyzed by tandem mass spectrometry. [M-H]⁻ ions were produced by continuous flow fast atom bombardment, and collision-induced dissociation of these ions was studied by using a triple quadrupole instrument. The product ion spectra obtained were characteristic of the structure of LTE₄, and mechanisms of ion formation were investigated by using deuterated compounds. β -Elimination of the peptide portion of LTE₄ by loss of CO₂ and ethylene amine leaves the C-1 carboxyl group ionized in the most abundant fragment ion for LTE₄ and all metabolites. Tandem mass spectrometry of fast atom bombardment-generated anions from ω - and β -oxidized metabolites of LTE₄ produced similar ions with only a minor influence of the third carboxyl group at the omega terminus evident. Tandem mass spectrometry was used to identify unequivocally the presence of unmodified LTE₄ in a high performance liquid chromatography-purified fraction of urine from a normal healthy volunteer after infusion with LTE₄. (*J Am Soc Mass Spectrom* 1991, 2, 314-321)

Sulfidopeptide leukotrienes are metabolites of arachidonic acid synthesized typically by inflammatory cells such as eosinophils, monocytes, macrophages, and mast cells. Activation of 5-lipoxygenase in these cells leads to production of a reactive intermediate leukotriene (LT)A₄ [1, 2]. This reactive intermediate is conjugated with the tripeptide glutathione to form the first member of the sulfidopeptide leukotrienes, LTC₄ [3]. LTC₄ has potent biological activities that cause constriction of various smooth muscles as well as edema in vascular beds [4]. These biological properties support the hypothesis that such leukotrienes serve as lipid mediators for many inflammatory and acute hypersensitivity reactions in animals and humans [5]. LTC₄ is known to be metabolized via sequential peptide cleavage reactions that lead to other biologically active members of the sulfidopeptide leukotriene class, LTD₄ and LTE₄, with the least active being LTE₄ [6]. Further metabolism of sulfidopeptide leukotrienes is known to take place. For example, LTE₄ is metabolized by the rodent to N-acetyl-LTE₄ [7] as well as ω - and β -oxidized products of the methyl terminus of the eicosanoid hydro-

carbon chain [8, 9]. Recently, human metabolites of LTE₄ excreted into urine were found to include 20-COOH-LTE₄, 18-COOH-LTE₄, 16-COOH- Δ^{13} -LTE₄, 16-COOH-LTE₃, and 14-COOH-LTE₃ [10]. The identification and measurement of these metabolites in urine may provide an index of in vivo production of sulfidopeptide leukotrienes.

The analysis of sulfidopeptide leukotrienes has centered around several strategies. Analysis by electron ionization mass spectrometry [11] and desorption chemical ionization mass spectrometry [12] have been reported, but extensive fragmentation and/or thermal decomposition of the molecule appeared to occur in spite of prior derivatization. Specific chemical degradation of the sulfidopeptide leukotrienes has been used to analyze these molecules by gas chromatography/mass spectrometry (GC/MS) [13]. Although the GC/MS technique is sensitive, a certain amount of structural information is unfortunately lost through degradation of the molecule. Direct analysis of these molecules has been accomplished with fast atom bombardment mass spectrometry (FAB/MS) [14].

Recently, analysis by tandem mass spectrometry of both positive and negative ions generated by FAB of several sulfidopeptide leukotrienes was reported [15] that provided specific data on the mass spectrometric behavior of these complex lipids. Collision-induced decomposition of both positive and negative ions of

* Current address: Institute of Pharmacological Sciences, University of Milan, Via Balzaretti 9, 20133 Milan, Italy.

Address reprint requests to Dr. Robert C. Murphy, Goodman Building, Room K929, National Jewish Center for Immunology and Respiratory Medicine, 1400 Jackson Street, Denver, CO 80206.

LTC₄ and LTE₄ suggested specific decomposition pathways for each species. These pathways were supported through the use of deuterium-labeled material. However, analysis of the ω - and β -oxidized metabolites of LTE₄ have not been described. All members of the sulfidopeptide leukotriene family have at least two carboxylic acid moieties and can readily localize a negative charge as a carboxylate anion. Our work is focused on studies of the decomposition of the abundant negative ions generated by FAB/MS of LTE₄ and its metabolites.

Methods

Materials. LTE₄ and N-acetyl-LTE₄ were obtained from Cayman Chemical (Ann Arbor, MI). Synthetic (20-²H₃)-LTE₄ was obtained from Biomol Laboratories (Philadelphia, PA). 20-COOH-LTE₄, 18-COOH-LTE₄, and 16-COOH-LTE₃ were obtained from Oxford Chemicals (Ann Arbor, MI). Each of these synthetic materials was purified by reverse phase high performance liquid chromatography (HPLC) by using an isocratic methanol/water system at pH 6 with 0.02% acetic acid. The content of methanol was adjusted to 55%, 45%, and 30% for each of the three ω -oxidized LTE₄ metabolites, respectively. (O-²H₃)-glycerol (98 atom % excess) was obtained from Aldrich Chemical Company (Milwaukee, WI). [³⁵S]LTC₄ was prepared by transcellular biosynthesis from LTA₄ by using [³⁵S]glutathione-labeled platelets as previously described [10]. Briefly, platelets were incubated for 3 h with 1 mCi of L-[³⁵S]cysteine then incubated with the free acid of LTA₄ (10 μ M) for 30 min at 37 °C. At the end of incubation, γ -glutamyltranspeptidase and leucine aminopeptidase were added to cleave the glutathione residue of LTC₄ to cysteine (LTE₄). The supernatant fraction was purified by reverse phase HPLC to collect [³⁵S]LTE₄.

Preparation of N-acetyl derivatives. N-acetyl derivatives of LTE₄, LTD₄, LTC₄, 20-COOH-LTE₄, 18-COOH-LTE₄, and 16-COOH-LTE₃ were prepared by dissolving the corresponding nonacetylated compounds (2 μ g) in 150 μ L of ethanol followed by treatment with 150 μ L NaHCO₃ (80 mM), 150 μ L triethylamine (80 mM), and 10 μ L acetic anhydride (10% in acetonitrile). The reaction was allowed to proceed for 30 min at 4 °C; after addition of 2 mL of water, the N-acetylated compounds were extracted using a C-18 solid-phase extraction cartridge and purified by reverse-phase HPLC by using the same system used for nonacetylated compounds.

Preparation of (N, O-²H₅)-LTE₄. LTE₄ contains five protons that readily exchange protons with water and protic solvents. (N, O-²H₅)-LTE₄ was prepared by dissolving LTE₄ (1 μ g) in 20 μ L of a solvent system composed of CH₃O²H, ²H₂O and (O-²H₃)-glycerol

(4:1:0.1; v/v/v). This solvent system was used as the flowing FAB mobile-phase during the analysis of (N, O-²H₅)-LTE₄ to prevent back-exchange of deuterons. Analysis by flowing FAB revealed an ion at m/z 442 [M-²H]⁻, indicating formation of (N, O-²H₅)-LTE₄.

Mass spectrometry. Continuous flow FAB/MS (CF-FAB/MS) was carried out on a Finnigan TSQ70 (San Jose, CA) by using the commercial ion source and saddle field-type ion gun. Xenon was used as particle source with the gun operated at 1 mA and 7-8 kV. Argon was used in the collision region with the offset potential of the second quadrupole at 20-30 eV. The pressure of argon in this second quadrupole field was adjusted to approximately 0.5 mtorr, which caused a 50% drop in the intensity of the ion at m/z 275 from the glycerol matrix. The matrix employed for CF-FAB/MS was methanol/water/glycerol (4:1:0.1) at 6 μ L/min. The ion source was maintained at 30 °C and the manifold of the mass spectrometer at 50 °C. The spectra were obtained by using the Finnigan data system. Typically six to ten scans were obtained during the elution of the sulfidopeptide leukotriene following injection (1 μ L) through a Rheodyne 7520 injector (Berkeley, CA). The spectra obtained were averaged and background subtracted with an averaged background spectrum.

Infusion of labeled LTE₄. Biosynthetically prepared [³⁵S]LTE₄ was infused into one human subject in a protocol approved by the Human Research Committee (University of Colorado Health Sciences Center) as previously described [10]. Briefly, [³⁵S]LTE₄ (4 μ Ci, 45 μ g) was infused in the right hand dorsal digital vein over a 10-min period through a 0.22 μ Millex sterile filter (Millipore, Bedford, MA). Urine samples were collected at 2, 5, 8, 15, and 24 h after infusion. Details concerning the recovery of radiolabeled metabolites in urine are discussed elsewhere [10]. Approximately 75% of that radioactivity eliminated in urine during the first 2 h following the beginning of the infusion of [³⁵S]LTE₄ was retained on a XAD-8 column after acidification of urine to pH 4 with formic acid. After washing the column with 200 mL of water, the radioactivity was eluted with 200 mL of methanol. This methanol eluate was dried under reduced pressure and reconstituted in 15% methanol/water and injected onto reverse-phase HPLC column (Ultrasphere ODS, 4 μ m, 0.46 $\times$ 25 cm, Beckman, San Ramon, CA) by using a linear gradient from 16 mM ammonium formate adjusted to pH 4.8 with HCl to methanol/water/acetic acid (90:10:0.1, v/v/v) over 55 min. The majority of the radioactivity eluted from the column between 60 and 62 min as a single large peak [10]. The HPLC fraction collected during this period was concentrated at reduced pressure and reconstituted in a small volume (10 μ L) for FAB/MS.

In preliminary studies with commercially available [^3H]LTE $_4$, it was found that LTE $_4$ was stable in urine for periods up to several hours at 37 °C and for several months at -20 °C in methanol/water solutions. However, this urine sample was immediately purified by XAD-8 extraction following collection to minimize any decomposition.

Results

Collision-induced dissociation of LTE $_4$. Low energy collision-induced dissociation (CID) of the abundant $[\text{M}-\text{H}]^-$ ions from the FAB of LTE $_4$ produced a wealth of fragment ions (Figure 1A). The most abundant masses observed were identical to those previously reported for the decomposition of LTE $_4$ in a tandem hybrid mass spectrometer [15]. Two isotopically labeled variants were available to probe ion structures which either had deuterons at each of the exchangeable proton positions (Figure 1B) or a perdeuterated methyl terminus (Figure 1C). Collision-induced dissociation of m/z 438 from LTE $_4$ $[\text{M}-\text{H}]^-$ results in the appearance of several abundant ions. The ion at m/z 420 is most likely a remote-site loss of a neutral molecule of water from the hydroxyl moiety at C-5. The most abundant ions appear to be formed following charge-driven processes with localization of the anionic site either on the C-1 carboxyl group or the cysteinyl carboxyl moiety. Several mechanisms can be drawn to rationalize the observed data that are a consequence of various sites of anion localization for the ion at m/z 438. However, considering the zwitterionic nature of α -amino acids in solution, one likely gas-phase negative ion species could, in fact, have three ionic sites that could decompose by β -elimination to structure **A** (Scheme I). The second mechanism illustrated involves a proton rearrangement in a favored six-membered ring remote from an ionized carboxyl moiety at C-1. Either process outlined in Scheme I involves a β -elimination mechanism with loss of two neutral species, ethylene amine and CO_2 (loss of 87 u) (**A**, m/z 351). The facile nature of these decomposition reactions may be due, in part, to the loss of the small neutral species as well as the stability of the carboxylate anion. Subsequent loss of water from this ion (**A**) yielded another abundant ion at m/z 333 (**B**), as shown in Scheme II. The abundant ion at m/z 235 (**C**) could arise from a charge-driven cleavage of the 5,6 bond in the arachidonate backbone of ion structure **A**, leaving the anionic site located on the carbon atom allylic to the conjugated triene (**C**; Scheme II). It is of interest that this ion was not observed in the previous study that used the tandem hybrid mass spectrometer [15]. When deuterium was incorporated into the exchangeable positions on OH, NH_2 , and COOH , m/z 235 was shifted to m/z 236, supporting migration of a single exchangeable proton to the sulfur atom. This ion retains the alkyl chain of

Figure 1. Fast atom bombardment mass spectrometry and collision-induced dissociation of the $[\text{M}-\text{H}]^-$ anions from (a) leukotriene (LT) E $_4$ at m/z 438; (b) $N,O\text{-}^2\text{H}_5$ -LTE $_4$ at m/z 442; and (c) $(20\text{-}^2\text{H}_3)$ -LTE $_4$ at m/z 441. The CID was performed by using argon in the collision quadrupole region at 20 eV collision energy (laboratory frame of reference).

LTE₄ as evidenced by shifting to m/z 238 in (20-²H₃)-LTE₄ (Figure 1C). A precursor scan of m/z 235 indicated that only m/z 351 and m/z 438 decompose to form this ion (Table 1). The abundant ion at m/z 333 (B) loses 44 mass units, again loss of CO₂, to form the ion at m/z 289 (D) (Scheme II). This was supported by precursor scan data (Table 1). The abundance of this ion may be related to the stability of the thiolate anion and delocalization of electrons over a nine atom system. A minor ion was observed at m/z 317, which would correspond to the loss of H₂S from A. This ion was also observed at m/z 317 (*N*, *O*-²H₅)-LTE₄ (Figure 1B) suggesting a triene epoxide structure shown in Scheme II, which has lost the exchangeable proton at the C-5 hydroxyl.

The minor ions at m/z 322 (E) and 120 (F) correspond to attack of a carboxylate anion on the proton of the C-5 hydroxyl group and the proton at C-5, respectively (Schemes III and IV). The stability of the product anion at m/z 322 is likely enhanced by facile transfer of the site of ionization to the carboxyl group (E') and loss of the stable aldehyde. This ion is shifted by 3 u in both deuterated species consistent with the structures E and E' (Scheme III). From the (*N*, *O*-²H₅)-LTE₄ study it is clear that the ion at m/z 120 contains only two deuteriums (m/z 122) from this labeled species (Scheme IV), which suggests that the

carboxyl proton in structure F originated from a non-deuterated position such as the proton at C-5. This is consistent with the spectrum from (20-²H₃)-LTE₄ (Figure 1C) in which m/z 120 is not shifted. The stability of this ion may be related to the stability of the thiolate anion.

The ion at m/z 115 (G) is quite interesting in that it contains no deuterium in any of the labeled species studied (Figure 1B and C). With the anionic site localized on the C-1 carboxyl, this ion could arise from a remote site fragmentation process with the proton on the hydroxyl at C-5 migrating to C-12, all double bonds shifting with cleavage of C-5-6 and formation of a carbonyl at C-5 (Scheme V). It could originate directly from [M-H]⁻ of LTE₄ as shown or also from m/z 351 based upon precursor scan data. Although the abundance of this ion is low, it was observed in LTE₄ species and metabolites (see below).

Collision-induced dissociation of LTE₄ metabolites. The CIDs of [M-H]⁻ generated by CF-FAB/MS of three synthetic metabolites of LTE₄ are shown in Figure 2.

Table 1. Precursor scan data obtained during negative ion fast atom bombardment mass spectrometry tandem of LTE₄

Observed ions (m/z)	Precursor ions ^b (m/z)
420	438
351 (A) ^c	438
333 (B)	438 420
235 (C)	438 351
289 (D)	438 420 351 333
317	438 351
120 (F)	438
115 (G)	438 351

^aPrecursor scan obtained by using triple quadrupole mass spectrometer.

^bIons observed in the product ion scan of m/z 438 are listed. Other ions found in these precursor scans corresponded to glycerol adducts.

^cDesignated ion structures shown in Schemes I-V.

Scheme V

Figure 2. Product ions spectra (tandem mass spectrometry) of the $[M-H]^-$ anions generated by fast atom bombardment with glycerol as matrix. (a) 20-COOH-LTE₄, m/z 468; (b) 18-COOH-LTE₄, m/z 440; and (c) 16-COOH-LTE₃, m/z 414. The CID was performed by using argon in the collision quadrupole region at 20-30 eV collision energy (laboratory frame of reference).

Very similar CID behavior was observed for these metabolites. The most abundant fragment ions in these decomposition spectra were again related to the loss of 87 u as described for LTE₄ and outlined in Scheme I as β -elimination. There was an additional series of ions evident in the CID spectra of the three metabolites studied that involve the loss of H₂S from ions C and D, previously discussed. With loss of H₂S,

the formation of a conjugated pentaene with the charge site localized on the ω -carboxylate position would result in ion (H) for the 20-COOH-LTE₄ metabolite observed at m/z 231 (Scheme VI). The loss of H₂S from anion D may result in formation of a conjugated hexaene (I) as shown in Scheme VII. As indicated, this loss of H₂S may be preceded by a proton rearrangement to protonate the thiolate anion, leaving a stable anion site at C-6 delocalized over a carbon π -system. These ions (I) at m/z 285, 257, and 231 were observed for each ω -carboxy metabolite, 20-COOH-LTE₄, 18-COOH-LTE₄, and 16-COOH-LTE₃, respectively.

Collision-induced dissociation of N-acetyl-LTE₄ metabolites. The metabolism of LTE₄ in the rat is dominated by N-acetylation of the free amino group on the cysteine portion of LTE₄ [7]. The CID mass spectrometry of N-acetyl-LTE₄ has been previously reported [15] and follows similar pathways to that described above for LTE₄ except that the acetylation of the amino group facilitates charge localization on the cysteine-carboxyl group. The mass spectra of N-acetyl-LTC₄/D₄/E₄ as well as the metabolites of LTE₄ observed in the rat, 20-COOH-N-acetyl-LTE₄, 18-COOH-N-acetyl-LTE₄, and 16-COOH-N-acetyl-LTE₃ are listed in Table 2. Similar ions discussed above were observed for the N-acetyl-derivatives with the most abundant ion (except for N-acetyl-LTD₄ and N-acetyl-LTC₄) being related to the formation of the carboxylate anion (A) (Scheme I) and loss of N-acetyl ethylene amine and CO₂.

Analysis for LTE₄ in human urine. When [³⁵S]LTE₄ (45 μ g) was injected into a human subject and urine was

Scheme VI

Scheme VII

Table 2. Negative ion tandem mass spectrometry of N-acetyl sulfidoepoxide leukotrienes. Collision-induced dissociation of [M-H]⁻ ions

Compound	[M-H] ⁻	[M-H-H ₂ O]	A	B	C	D	E	F	G	H	I	F-H ₂ S ^c
N-Ac-LTE ₄	480	462(1)	351(32)	333(29)	235(7)	289(4)	—	162(1)	115(10)	—	—	128(5)
N-Ac-LTD ₄	537	519(7)	351(3)	333(2)	—	289(1)	421(1)	219(15)	115(1)	—	—	185(40)
N-Ac-LTC ₄	666	648(4)	351(5)	—	—	—	—	—	—	—	—	314(20)
20-COOH-N-Ac-LTE ₄	510	492(4)	381(55)	363(20)	265(10)	319(5)	394(5)	162(1)	115(6)	231(8)	285(5)	296(25) ^d
18-COOH-N-Ac-LTE ₄	482	464(3)	353(25)	335(6)	237(5)	291(4)	366(2)	162(1)	115(2)	203(2)	257(3)	—
16-COOH-N-Ac-LTE ₃	456	438(6)	327(82)	309(20)	211(20)	265(10)	340(10)	162(1)	115(10)	177(5)	231(8)	—

^a Letters correspond to ion structures discussed in text for non-N-acetylated derivatives.^b Abundances are expressed relative to the observed parent ion.^c Other product ions with > 5% relative abundance.^d Loss of H₂O from m/z 314.

collected, a major portion of the radioactivity was eliminated into urine within the first collection period (2 h) [10]. Solid phase extraction of the first 2 h urine collection followed by reverse-phase HPLC purification resulted in isolation of a component that absorbed UV light with a UV maximum at 280 nm and shoulder maxima at 270 and 290 nm, typical for the conjugated triene chromophore of sulfidoepoxide leukotrienes [10]. From the intensity of the UV absorbance (molar extinction coefficient = 40,000) a total of no more than 400 ng was present in this fraction.

CF-FAB/MS analysis of an aliquot of the HPLC effluent (10%) at the elution time indicated by the UV absorption maximum produced the complex mass spectrum shown in Figure 3A. In addition to several other ions, there was an ion at m/z 438, suggesting the presence of LTE₄. Tandem mass spectrometry of this peak resulted in abundant product ions at m/z 235, 333, 351, and 420 (Figure 3B). The relative abundances of these ions were similar to that observed for the CID of authentic LTE₄ (Figure 1A). In addition to these ions, several other minor ions, whose structures were discussed above, were also apparent, including ions at m/z 115, 120, and 289. The tandem mass spectrometry experiment provided unequivocal evi-

Figure 3. The fast atom bombardment mass spectrum obtained from an aliquot of an HPLC-purified fraction of human urine collected following the infusion of LTE₄ into a human subject. (a) Continuous flow-fast atom bombardment mass spectrometry analysis of the fraction containing a radioactive and UV absorbing component corresponding to LTE₄, which has an [M-H]⁻ anion at m/z 438. (b) Tandem mass spectrometric analysis of the suspected [M-H]⁻ anion after collision-induced dissociation by using argon at 20 eV collision energy (laboratory frame of reference).

dence for identification of LTE_4 eliminated into urine by this human subject.

Discussion

Cysteine-containing leukotrienes analyzed by FAB/MS were shown to undergo facile CID reactions in the triple quadrupole mass spectrometer, leading to characteristic product ions. In most cases the processes involved in the formation of these product ions could be rationalized with a charge-driven mechanism from an initial localized charge at the cysteinyl carboxyl moiety followed by β -elimination or a charge-remote β -elimination reaction if the anionic site were localized at the C-1 carboxyl moiety. Many CID reactions appeared to involve attack of the carboxylate anion on a heteroatom-bonded proton at several sites in the LTE_4 structure. One typical characteristic of the decomposition pathways for these complex carboxylate anions was loss of small neutral molecules such as CO_2 , ethylene amine, H_2S , and H_2O .

It is of interest to note that the behavior of LTE_4 in low energy CID mass spectrometry is substantially different than that observed for LTC_4 and LTD_4 [15], possibly due to the influence of the additional amino acids in these structures. This suggests that each ionized group (at the pH of the matrix) greatly influences decomposition behavior and, furthermore, heterogeneity with respect to site-of-charge localization may exist in the $[\text{M-H}]^-$ ions. The results presented here as well as those previously reported [15] for CID, of sulfidopeptide leukotrienes are consistent with the cysteinyl leukotrienes decomposing by β -elimination to yield abundant thiol carboxylate anions, whereas glutathione leukotrienes yield the most abundant ions with negative charge retained by the peptide portion of the molecule. This behavior was seen with other glutathione and cysteinyl conjugates. Deterding et al. [16] found that N-acetyl cysteinyl conjugates of xenobiotics produced mostly thiolate anions $[\text{RS}]^-$ upon CID; whereas in negative ion FAB/CID of glutathione conjugates, the charge was retained by the glutathione moiety. Negative ion FAB/CID was used to screen for mercapturic acids $[\text{RSCH}_2\text{CH}(\text{HNAc})\text{COOH}]$ derived from various compounds. Regardless of the chemical nature of the conjugate, the dominant fragment was the alkyl thiolate anion $[\text{RS}]^-$ resulting from the loss of the amino acid portion [17].

The ω - β -oxidized metabolites of LTE_4 behaved in a similar fashion as parent LTE_4 in terms of their CID tandem mass spectrometry. Only a few ions appeared, implicating a role for the third carboxyl group in fragmentation processes of these molecules. The β -elimination process and other secondary fragmentations should prove useful in the identification of these metabolites of arachidonic acid in physiological fluids. As noted above, some decomposition ions for LTE_4

observed using the tandem quadrupole instrument were not reported when a hybrid tandem mass spectrometer was used [15]. The basis for such discrepancies is not known; however, the time-scale for those two experiments is somewhat different in terms of life-time of the anions as they enter the quadrupole collision chamber.

Tandem mass spectrometry of urine extracts of a human subject injected with LTE_4 provided strong evidence to support the occurrence of LTE_4 eliminated into urine, which is consistent with previous reports that used HPLC retention time data [7, 10, 18]. Because the amount of LTE_4 used for this analysis was less than 40 ng, this experiment illustrates the power of tandem mass spectrometry and CID/FAB/MS for the analysis of sulfidopeptide leukotrienes in physiologic fluids. Although the urine had been purified by reverse-phase HPLC, unequivocal identification of this molecule would not be possible based upon the appearance of a single ion in the CF-FAB mass spectrum. Conclusive evidence for the occurrence of this molecule was obtained through a combination of HPLC retention time, UV spectroscopy, and mass spectrometric techniques including CF-FAB/MS, CID, and tandem mass spectrometry. These results suggest the usefulness of this approach in the identification of human metabolites of LTE_4 in urine due to the unique behavior of these cysteine derivatives in the CID process.

Acknowledgment

This work was supported in part by a grant from the National Institutes of Health (HL25785).

References

- Samuelsson, B.; Funk, C. D. *J. Biol. Chem.* **1989**, *264*, 19469-19472.
- Samuelsson, B.; Dahlen, S. E.; Lindgren, J. A.; Rouzer, C. A. *Science* **1987**, *237*, 1171-1176.
- Murphy, R. C.; Hammarstrom, S.; Samuelsson, B. *Proc. Natl. Acad. Sci. USA* **1979**, *76*, 4275-4279.
- Piper, P. J. In *The Leukotrienes: Chemistry and Biology*, Chakrin, L. W., Bailey, D. M., Eds.; Academic: London, 1989; pp. 215-230.
- Samuelsson, B. *Science*, **1983**, *220*, 568-575.
- Lewis, R. A.; Austen, K. F. *J. Clin. Invest.* **1984**, *73*, 889-897.
- Keppler, D.; Hagman, W.; Rapp, S.; Denzlinger, C.; Koch, H. K. *Hepatology* **1985**, *5*, 883-891.
- Stene, D. O.; Murphy, R. C. *J. Biol. Chem.* **1988**, *263*, 2773-2778.
- Murphy, R. C.; Stene, D. O. *Ann. NY Acad. Sci.* **1988**, *524*, 35-42.
- Sala, A.; Voelkel, N.; MacLough, J.; Murphy, R. C. *J. Biol. Chem.* **1990**, *265*, 21771-21778.
- Morris, H. R.; Taylor, G. W.; Piper, P. J.; Tippins, J. R. *Nature* **1980**, *285*, 104-106.
- Murphy, R. C.; Clay, K. L.; Mathews, W. R. *Anal. Chem.* **1982**, *54*, 336-338.

13. Murphy, R. C.; Sala, A. In *Methods in Enzymology*; Murphy, R. C., Fitzpatrick, F. A., Eds.; Academic: New York, 1990; Vol. 187, pp. 90-98.
14. Murphy, R. C.; Mathews, W. R.; Rokach, J.; Fenselau, C. *Prostaglandins* **1982**, 23, 201-206.
15. Raftery, M. J.; Thorne, G. C.; Orkiszewski, R. S.; Gaskell, S. J. *Biomed. Environ. Mass Spectrom.* **1990**, 19, 465-474.
16. Deterding, L. J.; Srinivas, P.; Mahmood, N. A.; Burka, L. T.; Tomer, K. B. *Anal. Biochem.* **1989**, 183, 94-107.
17. Jones, A. D.; Winter, C. K.; Segall, H. J.; Buonarati, M. *Proc. Ann. Conf. Mass Spectrom.* **1988**, 36, 460-461.
18. Maltby, N. H.; Taylor, G. W.; Ritter, J. M.; More, K.; Fuller, R. W.; Dollery, C. T. J. *Allergy Clin. Immunol.* **1990**, 85, 3-9.