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Leukotriene Biosynthesis: Direct Chemical Ionization Mass Spectrometry of Underivatized Arachidonic Acid Metabolites

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An improved direct chemical ionization (DCI) mass spectrometric technique, using a polyimide-coated fused silica fiber as an extended probe tip, was used to obtain molecular ions and diagnostic fragment ions of underivatized arachidonic acid, 5-hydroperoxyeicosatetraenoic acid, 15-hydroperoxyeicosatetraenoic acid, leukotriene B_4 (LTB_4) and, for the first time, of leukotriene A_4 (LTA_4)-free acid. In this technique, sample compounds are coated onto the fused silica fiber and vaporized in the plume of the reagent gas plasma of a chemical ionization source without external heating of the probe. Both ammonia and isobutane DCI spectra were obtained for each compound. A volatile alkaline eluent system was developed that allowed reversed-phase high-performance liquid chromatography of LTA_4 to be followed rapidly by DCI mass spectrometry. With these techniques, the conversion of LTA_4 to LTB_4 during incubation with human liver microsomes was confirmed. Selected ion monitoring (SIM) of preselected ion fragments in the spectrum increases the selectivity of this technqiue and improves quantification in the range 100 ng to 10 pg.

INTRODUCTION

Leukotrienes are a group of endogenous compounds active in hypersensitivity and inflammations.[1,2] Following the appropriate stimulation to cause liberation of arachidonic acid by a phospholipase, a 5-lipoxygenase converts arachidonic acid into 5-hydroperoxy-eicosatetraenoic acid (5-HPETE), which is transformed by leukotriene A_4 (LTA_4) synthetase into LTA_4(5(*S*)-5, 6-oxido-7,9-*trans*-11-14-*cis*-eicosa-tetraenoic acid). In the presence of glutathione, glutathione-*S*-transferase converts LTA_4 into leukotriene C_4 (LTC_4), which can be further converted to leukotriene D_4 (LTD_4) and leukotriene E_4 (LTE_4) upon sequential peptidolysis.[3] Hydolysis of LTA_4 by LTA_4 hydrolases results in formation of leukotriene B_4 (LTB_4)(5(*S*),12(*R*)-dihydroxy-6,14-*cis*-8,10-*trans*-eicosatetraenoic acid), which at nanomolar concentrations causes chemotaxis of macrophages,[4] reduces peripheral blood flow,[5] induces degranulation and release of lysosomal enzymes from human polymorphonuclear leukocytes,[6] and exhibits regulatory functions in the maturation of T-lymphocytes mimicking those of interleukin-2.[7] Few reports describe the generation and metabolism of leukotrienes (in particular LTB_4) by other than specialized cells (i.e. macrophages, neutrophils, basophils). However, hepatocytes, too, seem capable of biosynthesis and inactivation of leukotrienes.[8,9] In the course of our work on the possible involvement of HPETEs or leukotrienes in hepatotoxic processes,[10] we required a method that allowed the structural elucidation of minimal amounts of these compounds after separation on high-performance liquid chromatography (HPLC).

Mass spectrometry has played an important role in the elucidation of the structure of leukotrienes and their precursors. However, low volatility and thermal instability of the compounds previously has required reduction of the hydroperoxides to alcohols followed by lengthy derivatizations to the silyl ether and methyl ester forms before gas chromatography/mass spectrometry (GC/MS).[11] Newer on- or off-line techniques employed in leukotriene analysis include thermospray liquid chromatography/mass spectrometry (LC/MS) and fast atom bombardment (FAB). Thermospray LC/MS was successfully applied in the structural analysis of 5-HETE and LTB_4 as well as the di- and trihydroxy metabolites of docosahexaenoic acid as produced by rat brain homogenates.[12] Additionally, the formation of 11-, 9- and 5-HETEs by human inflammatory cells was recently confirmed using the same technique.[13] FAB also has allowed successful analyses of underivatized LTC_4, LTD_4 and LTE_4, yielding high abundances of the molecular ions.[14,15] However, these techniques suffer from limitations such as restriction to mass spectrometry-compatible HPLC-eluent system, high background in the lower mass ranges, and the requirement of considerable amounts of material to be analyzed.

Direct chemical ionization (DCI) mass spectral analysis was first introduced by Baldwin and McLaf-

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ferty,[16] and has been widely applied to analysis of medium molecular weight polar compounds.[17] The application of the DCI technique by Schilling *et al.*[18] allowed the mass spectral analysis of underivatized prostaglandin H_2, a highly unstable endoperoxide intermediate in the prostaglandin biosynthetic pathway. Very recently, Jamieson *et al.*[19] presented an improvement to the implementation of the DCI technique by using a polyimide-coated fused silica fiber as an extended probe tip. Sample compounds are coated onto the fiber and vaporized in the plume of the reagent gas plasma of a chemical ionization source without external heating of the probe. In this report, Jamieson *et al.* reviewed the many contributions of other laboratories to the evolution of this DCI technique, and they discussed the mechanisms of sample vaporization from an unheated probe.

Here, we describe the use of this technique to obtain simultaneous information on both the molecular ion and diagnostic fragment ions of underivatized arachidonic acid, 5-HPETE, 15-HPETE, LTB_4 and, for the first time, of LTA_4-free acid. In addition, the conversion of LTA_4 to LTB_4 during incubation with human liver microsomes is confirmed. Selected ion monitoring (SIM) of a few preselected ion fragments in the spectrum increases the selectivity of this technique and improves quantification in the range 100 ng to 10 pg LTB_4.

EXPERIMENTAL

Materials

Arachidonic acid was purchased from Calbiochem, San Diego, California and was separated from autoxidation products using reversed-phase HPLC with methanol/water/acetic acid (90:10:0.02, v/v) as eluent. 5-HPETE and 15-HPETE were a generous gift by Dr C. C. Reddy, Pennsylvania State University; they were purified prior to use in methanol/water/acetic acid (83:17:0.02, v/v) on an Ultrasphere ODS HPLC column (C-18, 25 × 0.46 cm, 5 μm, Altex). Synthetic leukotriene A_4 methyl ester and leukotriene B_4 were kindly provided by Dr J. Rokach of Merck-Frosst Canada. All HPLC-grade solvents were purchased from Baker Chemical Company, Phillipsburg, New York. Triethylamine was obtained from Aldrich Chemical Company, Milwaukee, Wisconsin, and distilled prior to use. Other reagents were of highest purity available and used as received.

Preparation of LTA_4

Synthetic LTA_4 methyl ester (aliquots stored in hexane solution in liquid nitrogen) was thawed, the solvent evaporated under argon, and hydrolysis initiated in a 9:1 (v/v) mixture of methanol/50% (w/v) sodium hydroxide at 4 °C for 30 min. The hydrolyzate was then injected onto a reversed-phase HPLC column (PRP-1, 22 × 0.46 cm, 10 μm, Brownlee, operated with a guard cartridge). The column was eluted with an alkaline mobile phase (acetonitrile/water/triethylamine, pH 10.0, 30:70:0.002, (v/v) at a flow rate of 0.9 ml min^{-1}. LTA_4 was collected, the ultraviolet (UV) spectra recorded, and the LTA_4 concentration calculated using an $E_{281\,nm}$ of 40 000 M^{-1} cm^{-1}.[20] The sample was taken to dryness under argon at 4 °C, resuspended in cold acetonitrile, and immediately analyzed.

Biological generation of LTB_4 from LTA_4

Human liver microsomes were prepared essentially as described[20] and stored in 10 mM sodium were prepared essentially as described[20] and stored in 10 mM sodium borate, pH 8.5, containing 20% glycerol (v/v) and 5 mg BSA ml^{-1} in liquid nitrogen. Incubations were performed in the same buffer. Thus, microsomes (100 μl, 0.62 mg protein) were kept under a gentle flow of argon for 5 min at room temperature. LTA_4, previously prepared in acetonitrile/10 mM sodium borate, pH 10.0 (40:60, v/v) containing 5 mg BSA ml^{-1} [21] was added to 20 μM final concentration and the incubation continued for 2 min under argon. Reactions were stopped by adding 10 μl acetic acid. 500 μl methanol/water (2:3, v/v) containing 50 μg ml^{-1} prostaglandin B_1 were added and products were extracted into 3 ml diethyl ether. The organic phase was re-extracted in 1 ml distilled water, taken to dryness under argon, redissolved in 100 μl eluent (methanol/water/acetic acid, 75:25:0.02, v/v) and products separated isocratically on Ultrasphere ODS (C-18, 25 × 0.46 cm, 5 μm, Altex) at a flow rate of 0.9 ml min^{-1}. Material eluting with the same retention time as synthetic LTB_4 (i.e. 13.5 min) was collected, a UV spectrum recorded, the solution was taken to dryness under argon, resuspended in methanol, and stored at −20 °C until analysis.

Mass spectral analysis

Mass spectra were obtained by DCI as described by Jamieson *et al.*[19] Typically, 1 μl of a solution of 10 pg to 1 mg of the compound in an appropriate solvent (i.e. methanol or acetonitrile) was applied to the probe tip and the solvent was evaporated. The probe, which also introduced the reagent gas, was inserted into the mass spectrometer chemical ionization source, the sample was vaporized without heating the probe tip, and then ionized in the chemical ionization reagent gas plasma. Experiments were performed using a Hewlett Packard 5985A mass spectrometer scanned in the range of atomic mass units indicated every 50 ms

RESULTS AND DISCUSSION

DCI mass spectra of arachidonic acid, 5-HPETE, 15-HPETE, LTA_4 and LTB_4 were obtained using a DCI probe as described previously[19] and either ammonia or isobutane as reagent gases. We recorded spectra of 100 ng to 1 μg amounts of arachidonic acid, 5-HPETE, 15-HPETE and LTA_4, as well as of 10 ng biologically produced LTB_4, obtained after incubation of human liver microsomes with LTA_4. Using the SIM mode, however, resulted in the detection of as low as 10 pg synthetic LTB_4, and 100 pg 5- and 15-HPETE.

Mass spectral analysis of arachidonic acid, 5-HPETE and 15-HPETE

Figure 1 shows the DCI mass spectrum of arachidonic acid. When ammonia was used as a reagent gas, almost no fragmentation and no molecular ion were observed. However, a dominent ammonia molecular adduct occurred ($[M + NH_4]^+$, $m/z = 322$ (base peak) Fig. 1(a)). The fragment ion at m/z 346 is apparently due to an impurity in the arachidonic acid. Structural information could be obtained by substituting isobutane for ammonia as reagent gas (Fig. 1(b)). We suggest the following origins of some of the fragment ions detected with isobutane: $m/z = 287$ $[M + H - H_2O]^+$; and $m/z = 305$, the base peak $[M + H]^+$. The fragment ion at $m/z = 322$ is probably due to contamination of isobutane with ammonia in the inlet system.

The mass spectrum of 5-HPETE yielded a number of

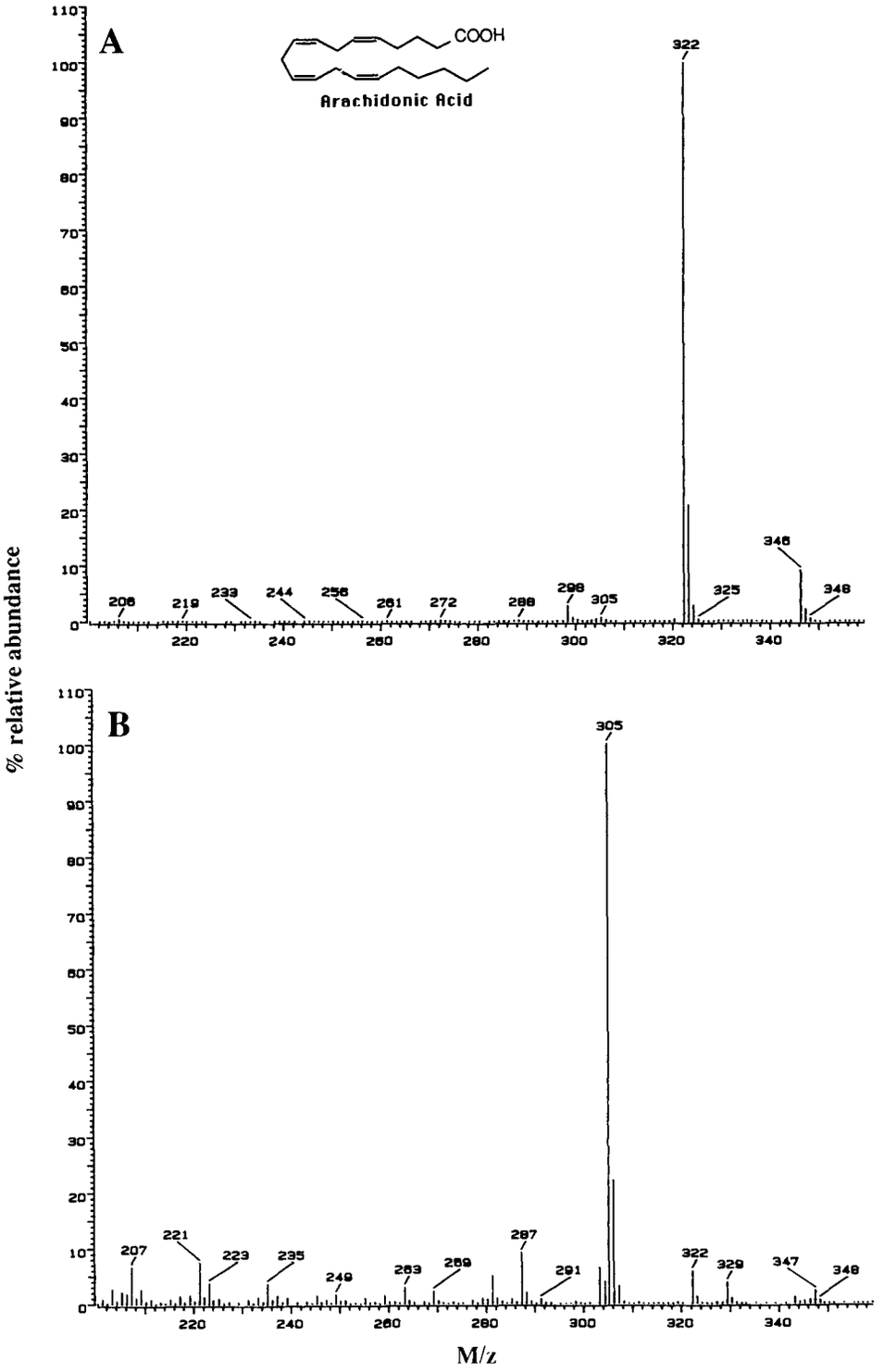

Figure 1. DCI mass spectra of underivatized arachidonic acid. 500 ng arachidonic acid were applied and masses recorded between 200 and 360 u. (a) Ammonia as reagent gas. (b) Isobutane as reagent gas. The source temperature was 300 °C.

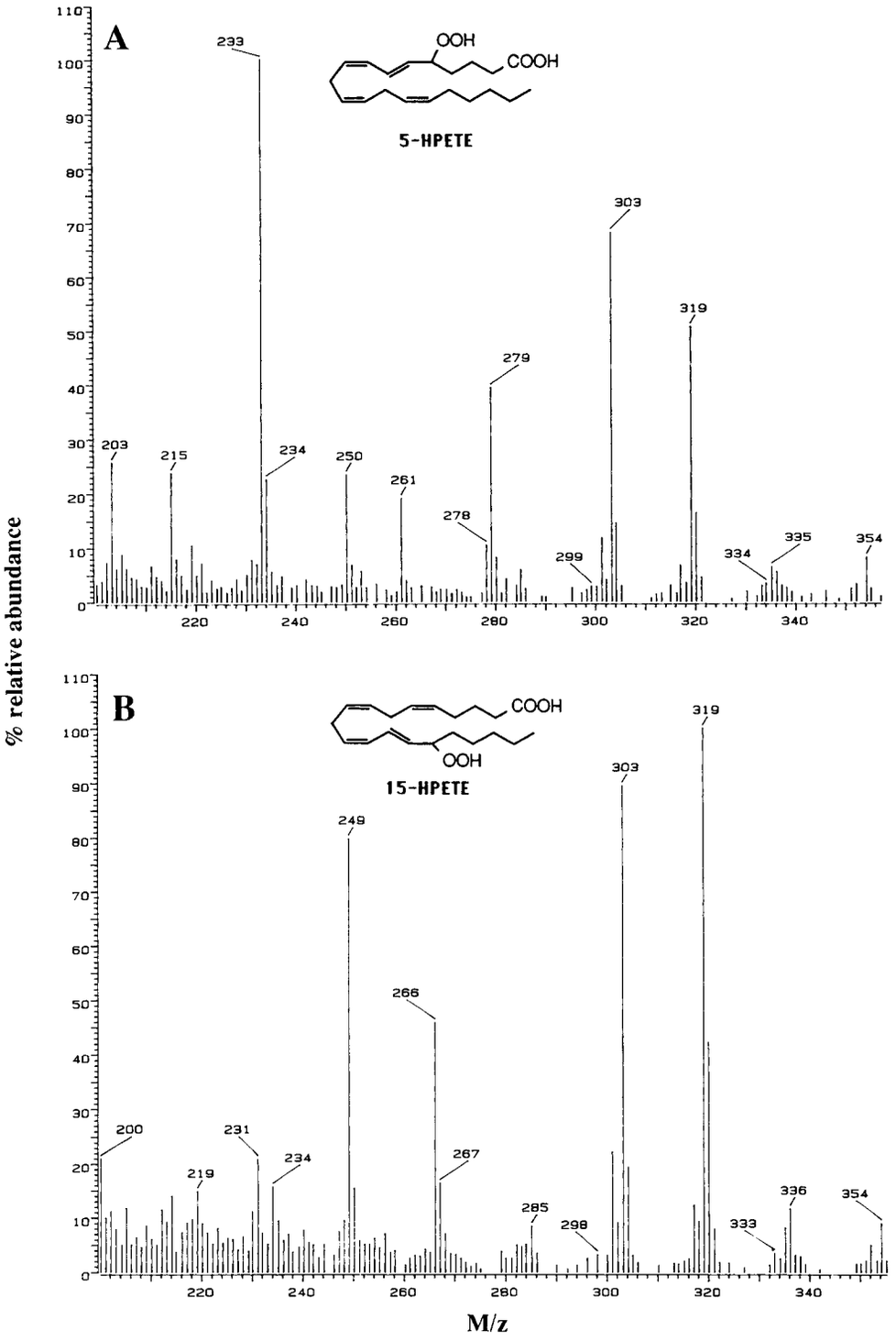

Figure 2. Ammonia DCI mass spectra of underivatized 5-HPETE (a) and 15-HPETE (b). 100 ng 5- or 15-HPETE were applied and masses recorded between 200 and 360 u. The source temperature was 300 °C.

diagnostically useful fragmentations, demonstrating the capacity of the DCI technique not only to yield information about the molecular weight of analyzed compounds, but also to distinguish, based on their distinct fragment ions, compounds of identifical relative molecular mass (RMM) (such as 5- and 15-HPETE as well as LTB_4, all with RMM = 336). This additional information is essential in the mass spectral analysis of these compounds and often not available in FAB techniques owing to high background noise in the lower mass range arising from ionization of the viscous matrix.[15]

Thus, using ammonia as reagent gas, an ammonia molecular adduct $[M + NH_4]^+$ wss obtained with 5-HPETE at $m/z = 354$ (Fig. 2(a)). Loss of water from the ammonium adduct yielded a weak signal at m/z 336 $[M + NH_4 - H_2O]^+$. The prominent fragment ions $m/z = 319$ and $m/z = 303$ are equivalent to a loss from the ammonia molecular adduct of $[NH_3 + H_2O]$ and $[NH_3 + H_2O_2]$, respectively. We suggest that the base peak ($m/z = 233$) arises by loss of C_5H_{10}, the terminal five carbon atoms on the fatty acid chain, from the m/z 303 fragment ion. The profiles of the ion currents in

Table 1. Characteristic fragment ions observed in the DCI spectra

Compound	Reagent gas	$[M-H]^+$	$[M+H]^+$	$[M+NH_4]^+$	Other
Arachidonic acid	NH_3			322(100)[a]	
	Isobutane		305(100)		287(9), 235(4), 207(7)
5-HPETE	NH_3			354(9)	336(6), 319(51), 303(77), 233(100)
	Isobutane		337(2)		319(14), 303(100), 285(13), 213(33)
15-HPETE	NH_3			354(8)	336(12), 319(100), 303(89), 266(46), 249(79)
	Isobutane		337(2)		319(52), 303(100), 285(16), 249(39), 231(61), 205(31)
LTA_4	NH_3		319(100)	336(9)	301(23), 256(11), 223(38), 188(33), 171(100)
	Isobutane	317(3)	319(6)		301(13), 223(23), 205(15), 171(15), 157(100), 141(99)
LTB_4	NH_3		337(12)	354(3)	336(33), 319(83), 301(100)
	Isobutane	335(3)			319(77), 301(100), 283(20), 257(29)

[a] Values in parentheses are per cent abundance relative to base peak (100%).

SIM mode of *m/z* 354, 319 and 303 were nearly identifical. The fragment ion observed at *m/z* 279 is due to a trace of dibutylphthalate in the sample.

Analysis of 15-HPETE under identifical conditions resulted in an ammonium molecular adduct, *m/z* 354, as well as fragment ions at *m/z* 336, 319 and 303 (Fig. 2(b)). However, the fragment ion at *m/z* 233 that was observed in the spectrum of 5-HPETE was absent in this spectrum, as would be expected based on the difference in structure of the two HPETEs. Instead, prominent fragment ions at *m/z* 266 and 249 were seen. The profiles of the ion currents in SIM mode of *m/z* 354, 319, 303 and 249 were nearly identifical.

Complementary information was obtained with both HPETEs using isobutane as a reagent gas (Table 1). However, in these spectra a protonated molecular ion ($M+H^+$) was observed instead of an ammonium molecular adduct. It is clear that SIM of *m/z* 233 and 249 would allow determination of the ratio of 5- and 15-HPETE, respectively, in mixtures of the two isomers.

Mass spectral analysis of LTA_4

To our knowledge, no mass spectra of underivatized LTA_4, the highly unstable allylic epoxide intermediate in the biosynthetic pathway of leukotrienes,[22] has been reported to date. In the course of our work on LTA_4 conversion to LTB_4 by human liver microsomes, we found it essential to confirm the structure and integrity of the free acid form of LTA_4 following hydrolysis of LTA_4 methyl ester. LTA_4 is supplied and stored as the more stable methyl ester. Before use, it must be hydrolyzed following published procedures.[21] We found that base hydrolysis did not result in a single pure product (~10% total impurities in three additional peaks on HPLC (Fig. 3(a)). In order to obtain pure LTA_4, we performed chromatography of the LTA_4 free acid on reversed-phase HPLC under alkaline conditions using acetonitrile/10 mM sodium borate, pH 10.0, 40:60 (v/v) as an eluent as previously described.[21] However, during mass spectral analysis, we encountered problems due to formation of a residue of sodium borate salts on the probe tip after evaporation of the sample. Therefore, we modified the reversed-phase HPLC eluent system in a way that allowed chromatography of LTA_4 under alkaline conditions (i.e. acetonitrile/water/triethylamine, pH 10.0, 30:70:0.002, v/v) in a completely volatile solvent system. Following chromatography, the free acid LTA_4 remained intact as judged by its UV spectrum (absorption triplet with relative maxima at 269, 280 and

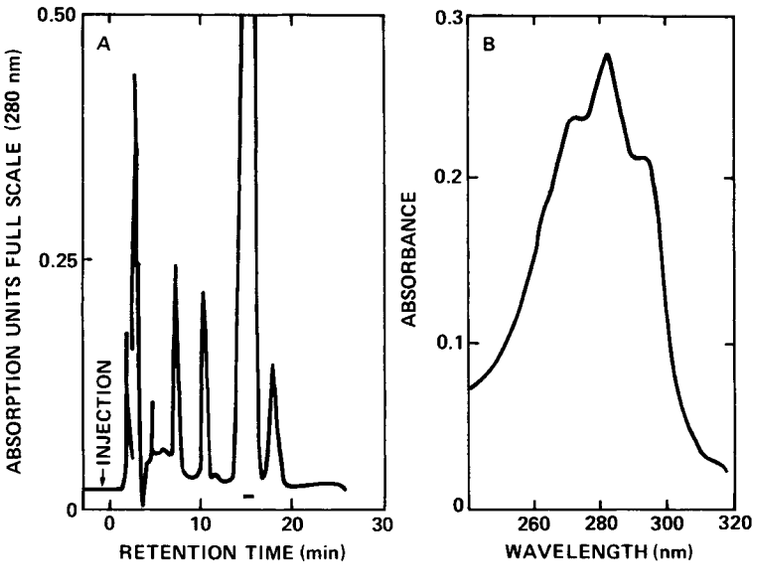

Figure 3. Reversed-phase HPLC profile and UV spectrum of LTA_4. (a) LTA_4 methyl ester (50 ng) was hydrolyzed as described in the Experimental section. The hydrolyzate was injected onto a reversed-phase HPLC column (PRP-1, 22 × 0.46 cm, 10 μm, Brownlee) and elution performed with acetonitrile/water/triethylamine, pH 10.0, 30:70:0.002 (v/v). The bar indicates the region where purified LTA_4 was collected. (b) The UV spectrum of the LTA collected under (a) was recorded in acetonitrile/water/triethylamine, pH 10.0, 30:70:0.02 (v/v).

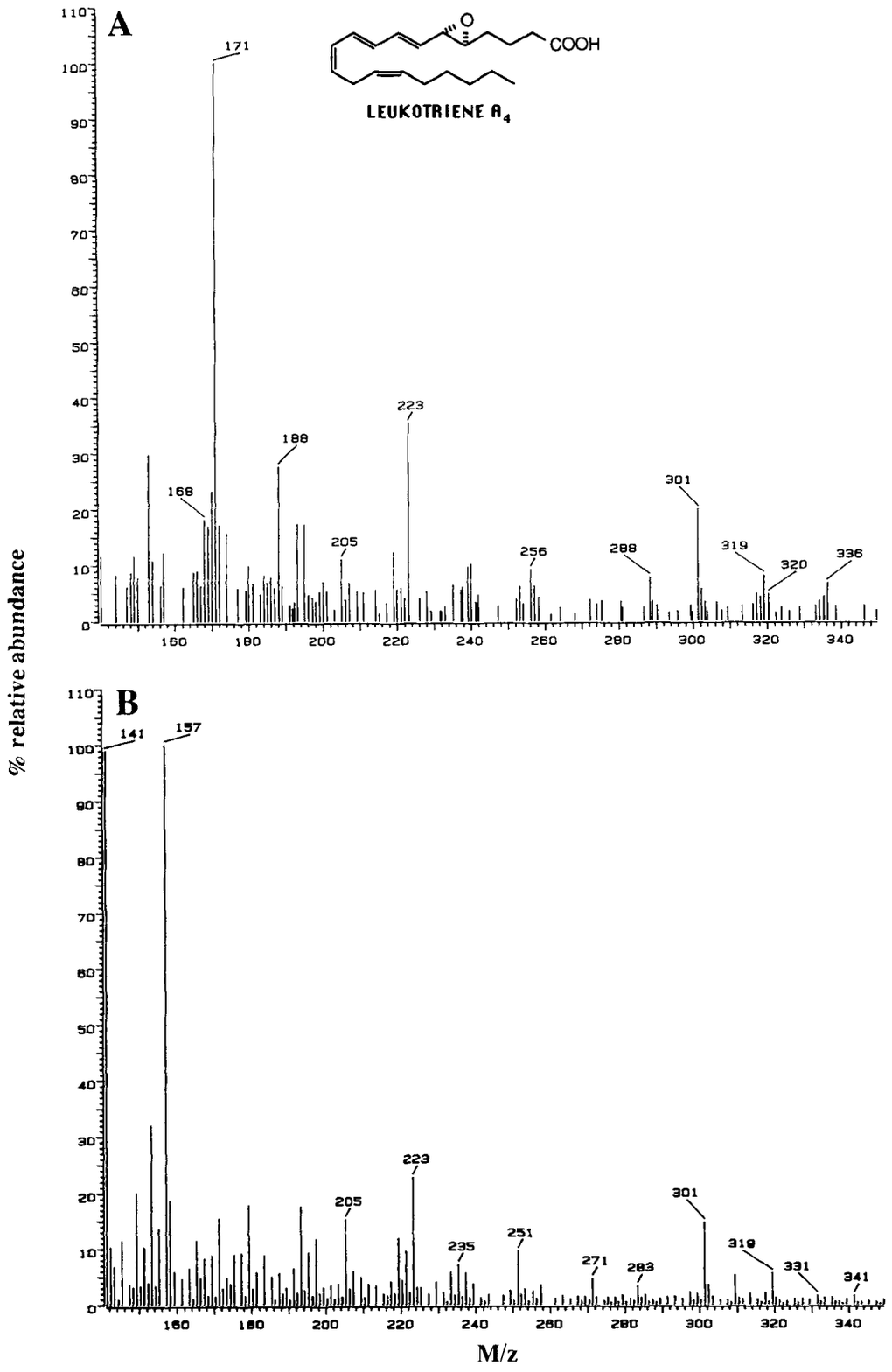

Figure 4. DCI mass spectra of underivatized LTA_4. 1 μg LTA_4, collected after reversed-phase HPLC (see Fig. 3) was applied and masses recorded between 140 and 350 u. The source temperature was 325 °C. Ammonia and isobutane were used as reagent gases in (a) and (b), respectively.

291 nm, Fig. 3(b)). If decomposition of LTA_4 occurs during chromatography or concentration, the decomposition products have an absorption spectrum that is a triplet with maxima at 260, 270 and 280. Therefore, the symmetry of the absorption spectrum shown in Fig. 3(b) is a good index of purity of LTA_4.

An ammonia molecular adduct ($[M + NH_4]^+$, $m/z = 336$) was easily detected in the DCI mass spectrum of this preparatively chromatographed, nonderivatized LTA_4 (Fig. 4(a)). The relative instability of the allylic epoxide can be estimated by comparing the background noise in this spectrum of 1 μg LTA_4 with

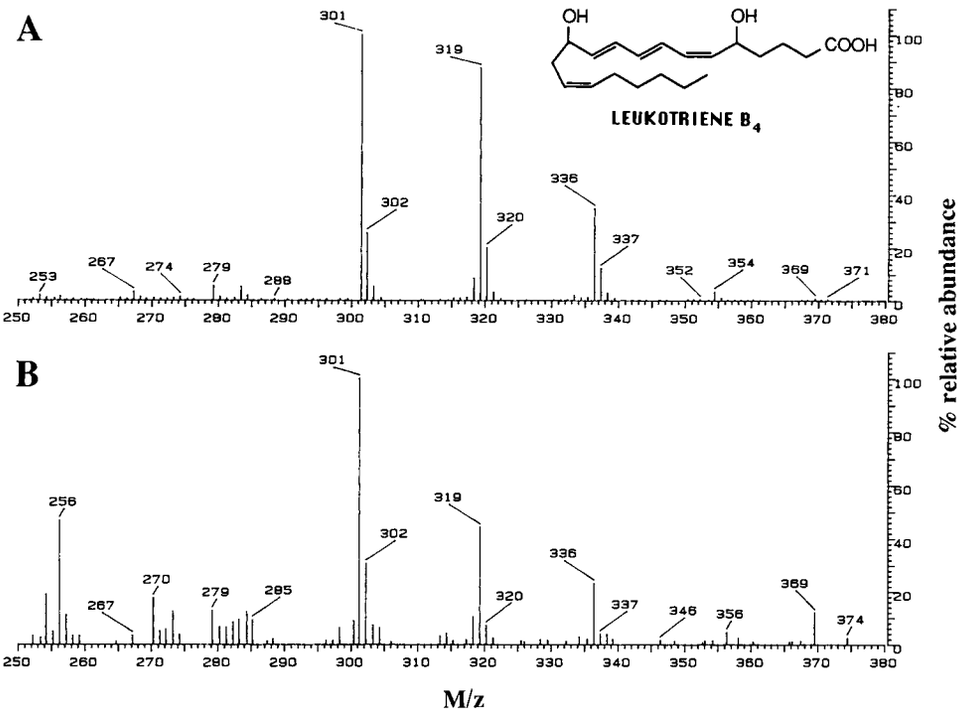

Figure 5. Ammonia DCI mass spectra of underivatized LTB_4. A mass range of 250–380 u was scanned at a source temperature of 300 °C. (a) 100 ng synthetic LTB_4 was applied. (b) 10 ng biologically derived material; three successive scans were summed.

that in Fig. 5(a) of only 100 ng LTB_4. A protonated molecule ion at $[M + H]^+$, $m/z = 319$, was also seen. Loss of water from the latter ion was attributed to $[M + H - H_2O]^+$, $m/z = 301$. A McLafferty rearrangement[23] of the epoxide is suggested to result in opening of the epoxide ring and migration of the C_7–C_8 and C_9–C_{10} double bonds followed by cleavage at C_9–C_{10}, providing the base peak $m/z = 171$ $[M + H - C_{11}H_{18}]^+$. Cleavage of the allylic C_{13}–C_{14} bond $[M + H - C_7H_{12}]^+$ gives rise to $m/z = 223$. The fragment ion at $m/z = 188$ can be attributed to the end of the hydrocarbon chain C_7 through C_{20}, $C_{13}H_{20}$, arising from the break of the allylic C_6–C_7 bond.

A slightly different fragmentation pattern was observed for LTA_4 with isobutane (Fig. 4(b)). Molecular ions due to both addition ($[M + H]^+$, $m/z = 319$) and loss of hydrogen ($[M - H]^+$, $m/z = 317$) were detected (Table 1). Allylic cleavage of the fatty acid chain at C_8–C_9, $[M + H - C_{12}H_{18}]^+$ could be attributed to $m/z = 157$, the base peak. A McLafferty rearrangement is suggested to result in the opening of the epoxide ring, migration of the C_7–C_8 double bond, followed by cleavage at C_7–C_8, providing $m/z = 141$ $[M + H - C_{13}H_{22}]^+$.

The isolation of LTA_4 from incubations of 5-HPETE with cell fractions[24] and its structural analysis without derivatization now seems feasible. However, the instability of LTA_4 under acid conditions poses a major problem in deproteinizing those incubations, and much work is still needed to apply this attractive mass spectral analysis to biologically generated LTA_4.

Mass spectral analysis of LTB_4

The major peaks in the ammonia chemical ionization mass spectrum of 50 ng underivatized LTB_4 (Fig. 5(a)) were: $m/z = 354$ $[M + NH_4]^+$; $m/z = 336$ $[M + NH_4 - H_2O]^+$; $m/z = 319$ $[M + H - H_2O]^+$; and a base peak of $m/z = 301$ $[M + H - 2H_2O]^+$. The ratio of peaks m/z 354, 336, 319, 301 in the spectrum of synthetic LTB_4 was 1.8:35:62:100. The appearance time of these four peaks during the vaporization process from synthetic LTB_4 was almost simultaneous. However, a control experiment revealed that during the vaporization process the ratios of the intensities of the three fragment ions with respect to the ammonia molecular adduct tended to increase with increasing scan number. The observed small changes in peak intensity ratios were probably due to thermal dehydration from the molecular ion with increasing ion source residence time. No further changes were observed in the latest scans (data not shown). Based on the intensity of the peaks m/z 336, 319 and 301 scanned in the SIM mode with 100, 10, 1, 0.1 and 0.01 ng synthetic LTB_4, we would be able to detect as little as 10 pg LTB_4.

LTA_4 conversion to LTB_4

In the course of our work aimed at the elucidation of the possible involvement of HPETEs and leukotrienes in hepatotoxic processes, the utility of DCI mass spectrometry in defining the leukotriene biosynthetic pathway in liver organelles was demonstrated by the following set of experiments. Human liver microsomes were incubated with LTA_4 for 2 min under argon and the reaction products were extracted and separated on reversed-phase HPLC as described in the Experimental section. The material eluting with the same retention time as synthetic LTB_4 (i.e. 13.5 min) was collected, a UV spectrum was recorded in order to compare the spectral identity of the biologically produced material

with that of synthetic LTB_4, and a DCI mass spectrum of this material was obtained (Fig. 5(b)). The major peaks *m/z* 354, 336, 319 and 301 were observed by SIM. The ratio of these peaks was 1.4:23:44:100 in the 10-ng sample isolated from LTA_4 incubations with human liver microsomes, thereby approaching that observed in the latest scans of synthetic LTB_4 (see above). In separate experiments it has been shown that the biological material has a binding affinity similar to authentic LTB_4 in a specific receptor displacement assay.[25] The mass spectral findings together with the high activity of this material in a receptor displacement assay for LTB_4 allow the assignment of the structure of LTB_4 to the biological material.

CONCLUSIONS

HPLC followed by DCI mass spectrometry is a useful tool for the analysis of oxygenated arachidonic acid metabolites for two reasons. First, the method allows a mass spectral analysis without time-consuming methylation and silylation procedures. Second, as opposed to HPLC/thermospray mass spectrometry, the HPLC technique is not restricted to eluent systems compatible with thermospray interfaces and therefore allows the use of optimal separation conditions. An HPLC buffer system of acetonitrile/H_2O/triethylamine was shown to stabilize free LTA_4 during chromatography and to be completely volatile when applied to the DCI probe. The DCI technique, using a polyimide-coated fused-silica extended probe tip, not only yields protonated molecule ions and molecular adducts with the respective reagent gases, but also provides simple yet informative fragmentation of the molecular adducts which allows identification of different structures of the same molecular weight. Ammonia and isobutane DCI mass spectrometry was shown to produce spectra of arachidonic acid and its derivatives involved in the biosynthesis of leukotrienes, including the very labile allylic epoxide intermediate LTA_4, without the need for derivatization. These techniques should prove useful in the structural analysis of subnanogram amounts of a broad variety of oxygenated arachidonic acid-derived metabolites produced by biological systems *in vitro* and model systems *in vitro*.

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REFERENCES

1. B. Samuelsson, *Science* **220**, 568 (1983).
2. S. Hammarstrom, *Ann. Rev. Biochem.* **52**, 355 (1983).
3. R. A. Lewis, K. F. Austen, J. M. Drazen, D. A. Clark, A. Marfat and E. J. Corey, *Proc. Natl. Acad. Sci. USA* **77**, 3710 (1980).
4. I. Hafstrom, J. Palmblad, C. L. Malmsten, O. Radmark and B. Samuelsson, *FEBS Lett.* **130**, 146 (1981).
5. T. J. Williams and P. J. Piper, *Prostaglandins* **19**, 779 (1980).
6. J. Palmblad, C. L. Malmsten, A. M. Uden, O. Radmark, L. Engstedt and B. Samuelsson, *Blood* **58**, 658 (1981).
7. H. M. Johnson and B. A. Torres, *J. Immunol.* **132**, 413 (1984).
8. F. J. Roll, D. M. Bissell and H. D. Perez, *Biochem. Biophys. Res. Commun.* **137**, 688 (1986).
9. J. Gut, A. K. Costa and J. R. Trudell, *Biochim. Biophys. Acta* **878**, 194 (1986).
10. J. R. Trudell, M. Bendix and B. Bosterling, *Biochim. Biophys. Acta* **803**, 338 (1984).
11. R. J. Strife and R. C. Murphy, *Prostaglandins Leukotrienes Med.* **13**, 1 (1984).
12. J. A. Yergey, H. Y. Kim and N. Salem, *Anal. Chem.* **58**, 1344 (1986).
13. R. Richmond, S. R. Clarke, D. Watson, C. G. Chappell, C. T. Dollery and G. W. Taylor, *Biochim. Biophys. Acta* **881**, 159 (1986).
14. R. C. Murphy, *Spectra* **10**, 11 (1985).
15. R. C. Murphy, W. R. Mathews, J. Rokach and C. Fenselau, *Prostaglandins* **23**, 201 (1982).
16. M. A. Baldwin and F. W. McLafferty, *Org. Mass. Spectrom.* **7**, 7 (1973).
17. A. L. Burlingame, A. Dell and D. H. Russell, *Anal. Chem.* **54**, 363R (1982).
18. A. B. Schilling, I. M. Zulak, M. L. Puttemans, E. R. Hall and D. L. Venten, *Biomed. Environ. Mass Spectrom.* **13**, 545 (1986).
19. G. C. Jamieson, C. C. Reuter and W. L. Fitch, *Anal. Chem.* **57**, 121 (1985).
20. J. Gut, T. Catin, P. Dayer, T. Kronbach, U. Zanger and U. A. Meyer, *J. Biol. Chem.* **261**, 11734 (1986).
21. F. A. Fitzpatrick, D. R. Morton and M. A. Wynalda, *J. Biol. Chem.* **257**, 4680 (1982).
22. R. C. Murphy, S. Hammarstrom and B. Samuelsson, *Proc. Natl. Acad. Sci. USA* **76**, 4275 (1979).
23. H. Budzikiewicz, C. Djerassi and D. H. Williams, *Mass Spectrometry of Organic Compounds*, Holden-Day, San Fancisco (1963).
24. O. Radmark, T. Shimizu, F. Fitzpatrick and B. Samuelsson, *Biochim. Biophys. Acta* **792**, 324 (1984).
25. D. W. Goldman and E. J. Goetzl, *J. Exp. Med.* **159**, 1027 (1984).