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Capillary isotachophoretic determination of cysteinyl leukotrienes

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ABSTRACT

The cysteinyl leukotrienes (LTs) C_4, D_4 and E_4 are among the most potent lipid mediators of anaphylaxis and inflammation. A capillary isotachophoretic method is described for the determination of these cysteinyl LTs. The method is based on anionic separation and detection using UV (254 nm) and conductivity detectors. The total analysis time is of the order of 30 min. The limit of detection of the method was determined to be 0.5 nmol of LTE_4. Despite of similar chemical structures, all three cysteinyl LTs can be determined simultaneously.

INTRODUCTION

Cysteinyl leukotrienes (LTs) C_4, D_4 and E_4 (Fig. 1) are arachidonic acid metabolites formed via the 5-lipoxygenase pathway [1]. These endogenous substances are among the most potent lipid mediators of anaphylaxis and inflammation [1,2]. LTE_4 is the main cysteinyl LT metabolite in human urine [3]. Determination of LTE_4 can be accomplished by bioassay [4], radioimmunoassay [5,6], high-performance liquid chromatography (HPLC) with UV detection [5,7] and gas chromatography–mass spectrometry [8]. Recently Holloway and Battersby [9] and our group [10] have shown that capillary isotachophoresis is a useful method for the determination of glutathione (GSH) derivatives of aromatic and aliphatic electrophiles. As cysteinyl LTs are GSH derivatives of the epoxide LTA_4, we investigated whether capillary isotachophoresis (ITP) is applicable to their determination. This paper describes the capillary isotachophoretic determination of LTC_4, LTD_4 and LTE_4. The method is based on anionic separation and detection using UV and conductivity detectors.

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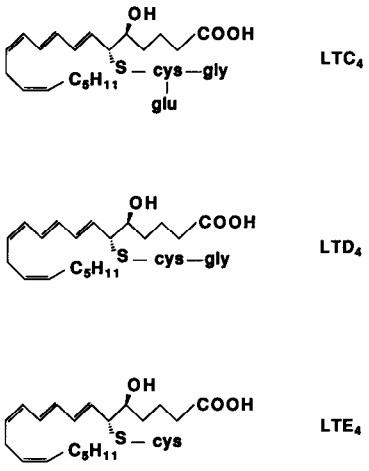

Fig. 1. Structures of the investigated cysteinyl leukotrienes LTC_4, LTD_4 and LTE_4.

EXPERIMENTAL

Isotachophoresis of LTC_4, LTD_4 and LTE_4 was performed on an LKB (Bromma, Sweden) Model 2127 Tachophor fitted with a polytetrafluoroethylene capillary (23 cm × 0.5 mm I.D.). Anionic analyses were performed with a leading electrolyte consisting of 5 m*M* hydrochloric acid, adjusted to pH 7.0 by the addition of Tris, and 0.25% (w/w) hydroxypropylmethylcellulose (HPMC) to reduce electro-endosmosis. The terminating electrolyte consisted of 10 m*M* phenol, adjusted to pH 10.0 by the addition of freshly prepared and filtered barium hydroxide solution. All analyses were carried out at room temperature. The zones were detected by UV (254 nm) and conductivity detectors. The isotachopherograms were recorded with an LKB 2120 line recorder at a chart speed of 0.5 mm/s. Analyses were carried out at a constant current of 25 μA. The terminator passed the detectors at a potential of about 10 kV. Injections were made with a 10-μl Hamilton syringe. The total analysis time was of the order of 30 min. The zones were measured by both UV and conductivity signals.

Cysteinyl leukotrienes were obtained from Paesel (Frankfurt, Germany) and used as received or purified by reversed-phase HPLC. Hydrochloric acid, barium hydroxide and phenol were purchased from Merck (Darmstadt, Germany), HPMC from Ega-Chemie (Steinheim, Germany) and Tris from Baker (Deventer, Netherlands).

RESULTS AND DISCUSSION

Fig. 2 shows an isotachopherogram from the analysis of a mixture of LTC_4, LTD_4 and LTE_4. All three cysteinyl LTs appear as UV-absorbing zones and can be completely separated by this ITP method. Specific zone lengths and reciprocal reference unit (RRU) values were determined by separate injection of each cysteinyl LT. The specific zone lengths for LTE_4, LTD_4 and LTC_4, obtained from the slopes of their calibration graphs, were determined to be 5.804, 6.12 and 6.35 s/nmol,

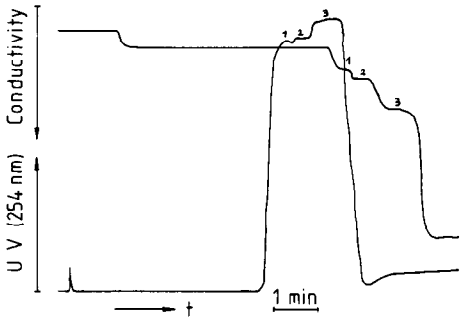

Fig. 2. Isotachopherogram for the separation of a mixture of (1) LTC_4 and (2) LTD_4, 4 nmol each, and (3) LTE_4, 10 nmol. For ITP conditions, see text.

respectively, which are lower than those of the GSH conjugates of aromatic electrophiles [10]. The RRU values of LTE_4, LTD_4 and LTC_4 estimated relative to the terminating ion from the relative step heights of the conductivity signal were determined to be (mean ± S.D.) 2.689 ± 0.085 ($n = 5$), 4.375 ± 0.112 ($n = 4$) and 5.185 ± 0.075 ($n = 4$), respectively. The corresponding RRU values of aromatic GSH conjugates are of the same order. The calibration graph for LTE_4 obtained by injection of 0.5–10 μl of a standard methanolic stock solution of LTE_4 was linear in the range 1–20 nmol and can be described by the regression equation $y = 4.489 + 5.804x$, $r^2 = 0.988$.

The sensitivity of the method is sufficient for the accurate detection of 0.5 nmol (220 ng) of LTE_4. This relatively high detection limit does not allow the application of this ITP technique to the determination of LTE_4 in human urine. However, the method could be useful for studying the formation and metabolism of cysteinyl LTs. Further, the on-line combination of ITP with mass spectrometry has recently been demonstrated for the first time by Udseth *et al.* [11], and has been shown to be suitable to the determination of analytes in the lower nanomolar range. ITP–mass spectrometry could allow more sensitive analysis and the determination of LTE_4 and other cysteinyl LTs in urine and other biological fluids using heavy stable isotope-labelled internal standards.

Because the method is applicable to cysteinyl LTs with closely similar structures, it could be an alternative technique for the analysis of all cysteinyl LTs, such as N-acetyl-LTE_4, the major cysteinyl LT metabolite in the urine of rat, and its metabolites of ω- and β-oxidation.

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REFERENCES

1 B. Samuelsson, *Science (Washington, D.C.)*, 220 (1983) 568.
2 R. A. Lewis and K. F. Austen, *J. Clin. Invest.*, 73 (1984) 889.
3 S. Hammarström, L. Örning, K. Bernström, B. Gustafsson, E. Norin and L. Kaijser, *Adv. Prostaglandin Thromboxane Leukotriene Res.*, 15 (1985) 185.

4 G. Hansson, S.-E. Dahlén and E. Granström, in F. Berti, G. Folco and G. P. Velo (Editor), *Leukotrienes and Prostacyclin*, Plenum Press, New York, 1983, p. 65.
5 S. Hammarström, *Annu. Rev. Biochem.*, 52 (1983) 355.
6 L. Levine, R. Morgan, R. A. Lewis, K. F. Austen, D. A. Clark, A. Marfat and E. J. Corey, *Proc. Natl. Acad. Sci. USA*, 78 (1981) 7692.
7 D. C. Henke, S. Kouzan and T. E. Eling, *Anal. Biochem.*, 140 (1984) 87.
8 M. Balazy and R. C. Murphy, *Anal. Chem.*, 58 (1986) 1098.
9 C. J. Holloway and R. V. Battersby, *Electrophoresis*, 7 (1986) 304.
10 D. Tsikas and G. Brunner, *J. Chromatogr.*, 470 (1989) 191.
11 H. R. Udseth, J. A. Loo and R. D. Smith, *Anal. Chem.*, 61 (1989) 228.