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LEUKOTRIENE A: STEREOCHEMISTRY AND ENZYMATIC CONVERSION TO LEUKOTRIENE B

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SUMMARY

Leukotriene A was assigned the structure 5(S)-trans-5,6-oxido-7,9-trans-11,14-cis-eicosatetraenoic acid by the enzymatic conversion of a synthetic product of known stereochemistry into the naturally occurring isomer of 5(S),12(R)-dihydroxy-6,8,10,14-eicosatetraenoic acid in human polymorphonuclear leukocytes.

INTRODUCTION

A new group of arachidonic acid derived metabolites (leukotrienes)(1) was recently discovered in polymorphonuclear leukocytes (PMNL) and mast cell tumor (MCT) cells. Thus, arachidonic acid after initial oxygenation at C-5 in a lipoxygenase catalyzed reaction was transformed into 5(S),12(R)-dihydroxy-eicosatetraenoic acid (leukotriene B, LTB) (2-5). This transformation was proposed to involve formation of a highly unstable epoxide intermediate, 5,6-oxido-7,9,11,14-eicosatetraenoic acid (leukotriene A, LTA) (6). The latter compound was also suggested to be a precursor of a slow reacting substance (SRS), leukotriene C (LTC) (1,7). The structure of LTC, 5(S)-hydroxy-6(R)-glutathiony 7,9-trans-11,14-cis-eicosatetraenoic acid, was recently eluci-

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dated (7,8). SRS is an important mediator in asthma and other immediate hypersensitivity reactions (9).

This paper describes the stereochemistry of LTA based on the enzymatic transformation of a synthetic product into LTB.

MATERIALS AND METHODS

Arachidonic acid was purchased from Nu-Chek Prep., Inc., Minnesota, USA. Ionophore A23187 was a kind gift from Dr. R.L. Hamill, Eli Lilly Research Laboratories, Indiana, USA. BW-755C (3-amino-1-(m-(trifluoromethyl)-phenyl)-2-pyrazoline), was a kind gift from Dr. F. Kuehl, Merck Institute for Therapeutic Research, Rahway, New Jersey, USA. Lymphoprep was purchased from Nyegaard & Co, As, Oslo, Norway, and Dextran T-500 from Pharmacia Fine Chemicals, Uppsala, Sweden.

Preparation of cell suspension

Suspensions of human PMNL were prepared from blood, obtained from healthy donors who had not taken any drugs for one week. The isolation was carried out as described in reference (4). The procedure consists of a) centrifugation to remove platelet-rich plasma, b) dextran-sedimentation, to separate white cells from red cells, c) ammonium chloride lysis to remove remaining red cells, d) gradient-centrifugation in Lymphoprep to separate different leukocytes. The resulting pellet contains mainly neutrophil and eosinophil granulocytes. Pellets were resuspended in PBS-buffer, pH 7.4 (30×10^6 cells/ml).

Synthetic LTA

An optically active aldehyde ester $[\alpha]_D^{25}$+ 68.6° (c=0.31 in chloroform), obtained by a multistep procedure from D-ribose, was converted by chain extension into 5(S)-trans-5,6-oxido-7,9-trans-11,14-cis-eicosatetraenoate (methyl ester, $[\alpha]_D^{25}$- 21.9 (c=0.32 in cyclohexane)). The synthesis will be described in detail elsewhere (10). All chemical intermediates were fully characterized by infrared, proton magnetic resonance and mass spectroscopy using chromatographically purified and homogenous samples.

Incubations

Synthetic LTA was added to 20 ml of a suspension of PMNL at 37°C to give a final concentration of 75 µM. After 10 min the incubation was stopped by addition of 30 ml of methanol. For use as reference cells from the same donor were incubated with arachidonate (150 µM) and ionophore A23187 (5 µM). In incubations with the inhibitor, BW-755C, the compound was added 5 min prior to the substrate at a concentration of 100 µM.

Extraction and column chromatography

The cell debris was spun down and washed with 10 ml of methanol. After acidification to pH 3 the combined medium and

methanol washing were extracted with ether. The ether extract was chromatographed on a 1 g silicic acid column (CC-4, Mallinckrodt) using ether-hexane (15/85, v/v) and ethyl acetate for elution.

Reverse phase high pressure liquid chromatography (RP-HPLC)

A column of Nucleosil C_{18} (Macherey-Nagel Co., Düren, West-Germany) was used with a Waters 6000A pump and a Waters U6K injector. A UV-detector (LDC-III) set at 280 nm was used to detect 5,12-dihydroxy-6,8,10,14-eicosatetraenoic acids and the 5,6-dihydroxy-7,9,11,14-eicosatetraenoic acids. The solvent used was methanol-water-acetic acid (75/25/0.01; v/v/v). Solvent flow was 1 ml/min. For complete separation of different isomers, the more polar mixture, methanol-water-acetic acid 70/30/0.01, was used. UV-spectra of separated components were recorded in methanol using a Cary 219 spectrophotometer.

Gas chromatography - mass spectrometry

An LKB 9000 instrument was used, equipped with an OV-101 column on Chromosorb W (H.P.) 100/120 Mesh. The temperature was 250°C, column length 3 m and inner diameter 2 mm. Helium flow was 20 ml/min. The energy of the ionisation-beam was 22.5 eV. Before gas chromatography, samples were converted to methyl esters by treatment with diazomethane in a mixture of ether and methanol, and silylated in a mixture of pyridine, hexamethyldisilazane and trimethylchlorosilane.

RESULTS

Incubation of synthetic LTA with a suspension of PMNL resulted in the formation of compounds I-V as shown by the RP-HPLC (Fig. 1). The chromatographic pattern was qualitatively

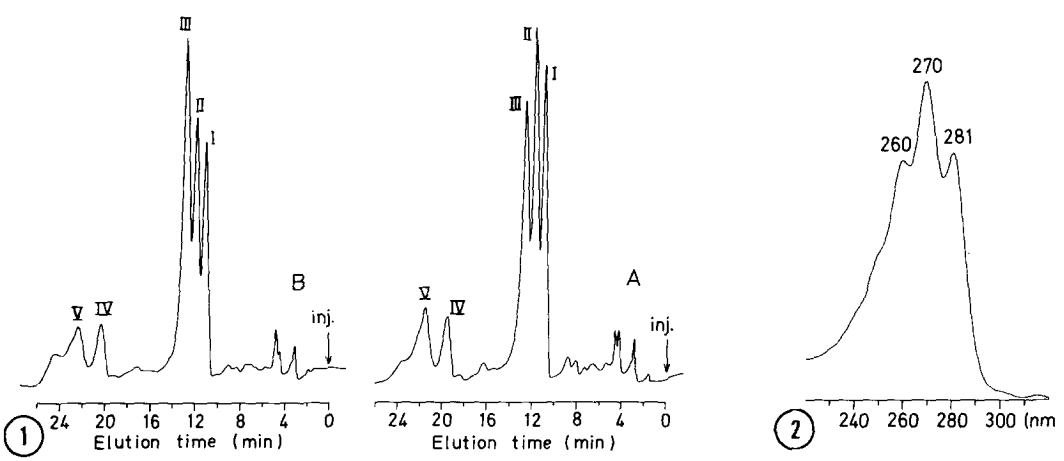

Fig. 1. Reverse phase HPLC chromatograms of products isolated from incubation of PMNL with 5(S)-trans-5,6-oxido-7,9-trans-11,14-eicosatetraenoic acid (1A), or with arachidonic acid and ionophore A23187 (1B. Solvent system: methanol/water/acetic acid, 75/25/0.01, v/v/v.

Fig. 2. UV-spectrum of the enzymatically formed isomer of 5(S), 12(R)-dihydroxy-eicosatetraenoic acid (compound III). Spectrum was recorded in methanol.

the same as when the cells were incubated with arachidonic acid and ionophore A23187. A control incubation without substrate showed no conversion to compounds I-V. Addition of the inhibitor BW-755C to another incubation gave the same results as without inhibitor demonstrating that the metabolites were not derived from endogenous arachidonic acid. The pyrazoline derivative (BW-755C), has been shown to inhibit formation of mono- and dihydroxyacids from arachidonic acid in PMNL (11). Also, no 5-hydroxyeicosatetraenoic acid could be detected in any of the incubations with synthetic LTA. The compounds formed from LTA were identified by their RP-HPLC characteristics, UV-spectra, gas chromatographic properties and mass spectra. Authentic metabolites formed from arachidonic acid by PMNL and characterized as described in detail earlier (3,5,6) were used as references. The data are summarized in Table I. The UV-spectrum of compound III formed enzymatically from synthetic LTA is shown in Fig. 2. The mass spectrum of compound III derived from synthetic LTA is shown in Fig. 3.

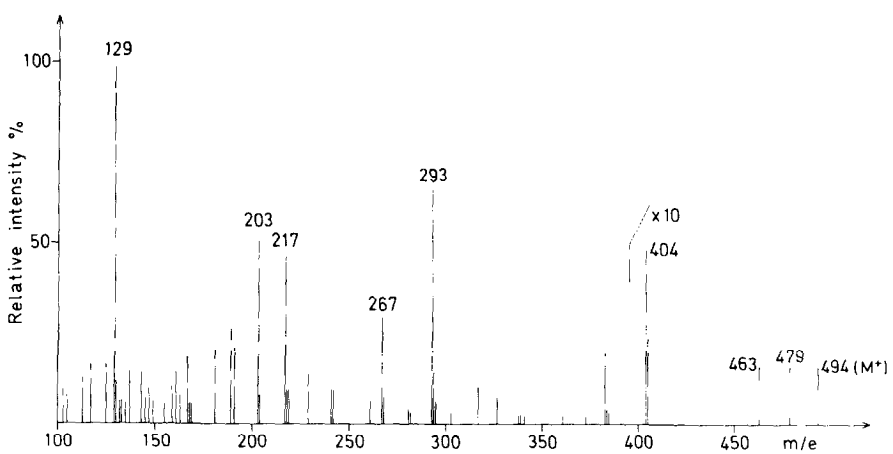

Fig. 3. Mass spectrum of the enzymatically formed isomer of 5(S),12(R)-dihydroxy-eicosatetraenoic acid (compound III). The C-value for this compound was 23.6.

TABLE 1. Analytical data for compounds I - V

	Compound I	Compound II	Compound III	Compound IV	Compound V
UV-maxima (nm)	259	259	260	263	263
	269	269	270	272	272
	280	280	281	283	283
C-value (OV-101)	24.8	24.8	23.6	23.8	23.8
Major ions in mass spectrum	494 M^+	494 M^+	494 M^+	494 M^+	494 M^+
	479	479	479	479	479
	463	463	463	463	463
	404	404	404	404	404
	293	293	293	291	291
	267	267	267	225	225
	217	217	216	203*	203*
	203	203	203	171	171
	129*	129*	129*		

* base peak

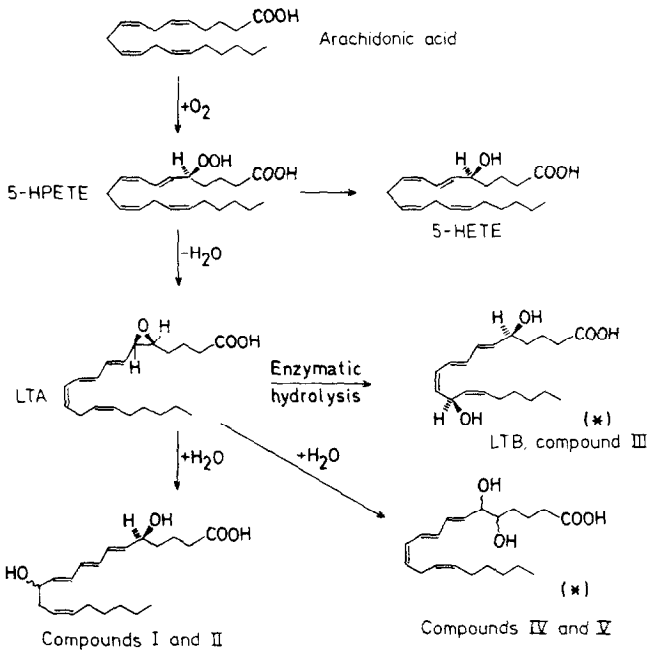

Fig. 4. Transformations of arachidonic acid and leukotrienes in human PMNL. * indicates that configuration of conjugated triene has not been determined.

DISCUSSION

Earlier studies on the transformation of arachidonic acid in polymorphonuclear leukocytes (PMNL) and mast cell tumor (MCT) cells to leukotriene B (LTB) and LTC indicated that an unstable epoxide, 5,6-oxido-7,9,11,14-eicosatetraenoic acid (LTA), was formed as intermediate (Fig. 4) (6,7). Racemic cis and trans-5,6-oxido-7,9-trans-11,14-cis-eicosatetraenoic acid was recently prepared (12) and found upon treatment with acid to give products with properties similar to those obtained by non-enzymatic acid catalyzed hydrolysis of enzymatically formed LTA (6,12).

In considerations of the stereochemistry of naturally occurring epoxide LTA it can be assumed that it is the 5(S)-epoxide since it originates in 5(S)-5-hydroperoxy-6-trans-8, 11,14-cis-eicosatetraenoic acid. Furthermore, the 11,14-diene, which is not involved in the transformation to LTA, should re-

tain its cis configuration. However, the cis/trans isomerism of the epoxide and the 7,9-diene was unknown.

A multistep synthesis starting with an optically active aldehyde ester derived from D-ribose, provided after chain extension 5(S)-trans-5,6-oxido-7,9-trans-11,14-cis-eicosatetraenoic acid. This compound was transformed enzymatically by human PMNL into the naturally occurring isomer of 5,12-dihydroxy-6,8,10,14-eicosatetraenoic acid (compound III or LTB). Two other isomeric 5,12-dihydroxy acids and two isomeric 5,6-dihydroxy acids, formed by non-enzymatic hydrolysis of the unstable epoxide intermediate (LTA) (6), were also identified as products of synthetic LTA. Only compounds I, II, IV and V were formed when the synthetic LTA was subjected to mild acid hydrolysis. The enzymatic transformation of synthetic LTA into the naturally occurring form of LTB strongly indicates that LTA is 5(S)-5,6-oxido-7,9-trans-11,14-cis-eicosatetraenoic acid.

The stereochemistry assigned to LTA is also consistent with a precursor-product relationship with naturally occurring LTC-1 as described in the accompanying report (13).

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