Conversion of 5,8,11-Eicosatrienoic Acid to Leukotrienes C₃ and D₃

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5,8,11-Eicosatrienoic acid was converted by mouse mastocytoma cells stimulated with ionophore A23187 to two slow reacting substances. These were characterized by spectroscopy and by chemical and enzymatic degradations as two geometric isomers of 5-hydroxy-6-S-glutathionyl-7,9,11-eicosatrienoic acid (C₄-D₄, C₄-E₄, C₄-E₄; 11-trans-leukotriene C₃). Corresponding cysteinylglycine compounds (leukotriene D₃ and 11-trans leukotriene D₃) were obtained from the leukotriene C₃ isomers by treatment with kidney y-glutamyl transpeptidase. The biological effects of leukotrienes C₃ and D₃, on the isolated guinea pig ileum, were approximately the same as the leukotrienes derived from arachidonic acid.

Slow reacting substance, first described in 1938 as a factor from lung which induces a characteristic contraction of guinea pig intestine (1, 2), appears to be an important mediator of bronchoconstriction in asthma (3). The structure of SRSₐ was published in 1979 (4, 5), and the stereochemistry was elucidated by total chemical synthesis (6) shortly thereafter (7). This compound, 5(S)-hydroxy-6(R)-S-glutathionyl-7,9-trans, 11,14-cis-eicosatetraenoic acid has been designated leukotriene C₃ (8). In addition the 11-trans isomer of leukotriene C₃ was formed in lesser amounts (9). Leukotriene C₃ is a potent stimulator of respiratory smooth muscle contractions in the guinea pig and in man (about 1000-fold more potent than histamine) and increases the insufflation pressure and the capillary permeability in the guinea pig (10). Leukotriene C₃ is converted to the corresponding cysteinylglycine derivative, leukotriene D₃ (8), by y-glutamyl transpeptidase (11, 12). Leukotriene D₃ has similar biological effects as leukotriene C₃ and is more potent in certain systems (10). It is the major component of SRS produced by rat basophilic leukemia (RBL-1) cells (11, 12) and of SRS from immunologically stimulated human (13, 14) and immunologically stimulated guinea pig (13, 14). In contrast, SRS from rat mononuclear cells consists of leukotriene C₄ and leukotriene D₃ (80 and 20%, respectively, on a molar basis (13, 15)). Some evidence for the formation of leukotriene D₃ by basophilic leukemia cells (16, 17) and immunologically stimulated guinea pig lung (18) has also been presented.

Recently, an analog of leukotriene C₃ containing an additional double bond was characterized (leukotriene C₃, (9)). The present paper describes the conversion of eicosatrienoic acid (n-9) to analogs of leukotrienes C₃ and D₃ as well as to 11-trans leukotriene C₄ and D₄ analogs, lacking the Δ⁴ double bond.

EXPERIMENTAL PROCEDURES

Materials—Ionophore A23187 and the SRS antagonist FPL 55712 (20) were kindly provided by R. Hamill of Eli Lilly and P. Hedquist, P. Hammarstrom, S. Samuelsson.

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The abbreviations used are: SRS, slow reacting substance; HPLC, high performance liquid chromatography.


RESULTS

Fig. 1 illustrates the effects of 5,8,11-eicosatrienoic acid (54 μM) on leukotriene formation by mastocytoma cells stimulated...
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with ionophore A23187 and L-cysteine. In the absence of exogenous fatty acid, leukotriene C₄ (4, 5, 8) and 11-trans leukotriene C₄ (9) were the major products (56 and 13 nmol/3·10⁹ cells, respectively). In the presence of eicosatrienoic acid lower amounts of leukotriene C₄ and 11-trans leukotriene C₄ were formed (15 and 5 nmol/3·10⁹ cells, respectively). Instead, two products with longer elution times (I and II) 31 and 8 nmol/3·10⁹ cells, respectively) appeared. In another experiment using 5·10⁹ mastocytoma cells and 50 μM eicosatrienoic acid the yields were 24 nmol of leukotriene C₄, 6 nmol of 11-trans leukotriene C₄, 32 nmol of compound I, and 9 nmol of compound II.

Compound I—The UV spectrum of compound I (Fig. 2) had an absorbance maximum at 279 nm and shoulders at 291 and 269 nm. Each of these values is approximately 1 nm lower than for the spectrum of leukotriene C₄ (4). As previously discussed (4) the spectrum indicates the presence of a conjugated triene with an allylic thioether. Desulfurization of compound I (30 nmol) with Raney nickel yielded 5-hydroxyeicosanoic acid as judged by gas-liquid chromatography-mass spectrometry (c value 21.5 (SE-30); ions at m/e 389 (M - 15, -CH₃), 367 (M - 15 - 32, -CH₂), 313 (M - 101, -CH₂(CH₂)₂COOCH₃), 203 (M - 211, (CH₃)₃SiO+.

Table 1: Elution times on HPLC and amino acid compositions of leukotrienes derived from eicosatrienoic acid (n-9)

<table>
<thead>
<tr>
<th>Compound</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Elution time</td>
<td>18.6</td>
<td>20.4</td>
<td>29.4</td>
<td>31.5</td>
</tr>
<tr>
<td>Amino acid composition</td>
<td>Glutamic acid</td>
<td>0.83</td>
<td>0.94</td>
<td>0.05</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.94</td>
<td>0.98</td>
<td>1.01</td>
<td>n.d.</td>
</tr>
<tr>
<td>Half-cystine</td>
<td>0.29</td>
<td>0.27</td>
<td>0.32</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

* Measured in minutes. Conditions for HPLC as in Fig. 1 except that the methanol to water ratio was 75:25, v/v.
* Measured in mol/mol. Samples were hydrolyzed with 6 N HCl for 21 h prior to the analyses. Other residues were not detected or were less than 0.07 mol/mol.
* n.d., not determined.
Amino acid analyses (Table I) gave similar results as for leukotriene C₄ (5) viz. approximately 1 mol of glutamic acid, 1 mol of glycine, and a smaller amount of cystine per mol of compound I. Compound I (6 nmol) was transformed by γ-glutamyl transpeptidase to a less polar derivative (compound III, 4 nmol) lacking glutamic acid (Table I). This suggested that glutamic acid was NH₂-terminal and attached as a γ-peptide in compound I. Hydrazinolysis showed that glycine was COOH-terminal, establishing that the peptide part of compound I was identical with that of leukotriene C₄ (glutathione). Compound I (90 nmol) was subjected to oxidative ozonolysis, and the products were converted to methyl ester derivatives and analyzed by gas-liquid chromatography-mass spectrometry (Fig. 3). Two products were identified by comparisons with authentic reference compounds as nonanoic acid (c value 9.0; ions at m/e 172 (M), 157 (M – 15, CH₃), 143, 141 (M – 31, O-CH₃), 129, 115, 101, 87 and 74) and 1,5-pentanedioic acid (c value 13.3 (EGSSX); ions at m/e 129 (M – 31, O-CH₃), 128, 101 (M – 59, COOCH₃)).

The structure of compound I was further substantiated by the formation of 1,5-pentanedioic acid during the oxidative ozonolysis (cf. discussion in Ref. 4). Compound I was not converted by soybean lipoxygenase to a 15-hydroperoxy derivative as judged by UV spectroscopy (Fig. 4). This is in agreement with the lack of a Δ¹⁴ double bond.

The structure of compound I is, therefore, 5-hydroxy-6-S-glutathionyl-7,9,11-eicosatrienoic acid (leukotriene C₃, Fig. 5). It is likely that the stereochemistry is identical with that of leukotriene C₄ (5(S),6(R)7,9-trans,11-cis (7)).

Compound II—The UV spectrum of compound II was shifted 2 nm hypsochromatically (λmax at 277 nm) compared to the spectrum of leukotriene C₄ (Fig. 2). This suggests the presence of an all trans triene chromophore (cf. Ref. 9). The amino acid composition (Table I) and the conversion of compound II by γ-glutamyl transpeptidase to a less polar derivative (Table I, compound IV) suggested that the peptide parts of compounds I and II were identical. The relative HPLC elution time of compound II compared to compound I was the same as the relative elution time of 11-trans leukotriene C₃ compared to leukotriene C₄ (1.1, Fig. 1). Based on this, compound II was tentatively identified as the 11-trans isomer of leukotriene C₃ (Fig. 5).

Compound III—Compound III was obtained by treatment of leukotriene C₃ with γ-glutamyl transpeptidase under conditions which lead to the conversion of leukotriene C₃ to leukotriene D₃ (11). The UV spectrum of compound III was identical with that of leukotriene C₃, and amino acid analyses showed that compound III lacked glutamic acid (Table I). The relative elution time of compound III compared to leukotriene C₃ (1.6; Table I) was similar to the relative elution time of leukotriene D₃ compared to leukotriene C₃ (1.7, cf. Ref. 12). Compound III is, therefore, the cysteinylglycine analog of leukotriene C₃ (Fig. 5, leukotriene D₃).

Compound IV—Based on its mode of formation and its elution time on HPLC, compound IV is the cysteinylglycine analog of 11-trans leukotriene C₃ (Fig. 5, 11-trans leukotriene D₃).

Conversion of [3H]-labeled Eicosatrienoic Acid to Leukotriene C₃—Fig. 6 shows an HPLC chromatogram obtained from an incubation of 3.4 mCi [5,6,8,9,11,12-3H]eicosatrienoic acid (62 Ci/mmol) with mouse mastocytoma cells (3.4 × 10⁶) in Tyrode's buffer before and after 45-min incubation at 23°C (4). Absorbance, 0-0.04 units.

FIG. 4. Effects of soybean lipoxygenase on the UV spectra of compound I (——) and leukotriene C₄ (—-). Spectra were recorded in Tyrode's buffer before and after 45-min incubation at 23°C (4). Absorbance, 0-0.04 units.

FIG. 5. Structures of leukotrienes formed from eicosatrienoic acid (n-9).
the presence of L-cysteine (10 mM) and A23187 (20 μM). Approximately 50 μCi of radioactive product with elution time 42 min were obtained. In another experiment the carrier-free acid was diluted with unlabeled eicosatrienoic acid (n-9) to 22 Ci/mol, and 0.21 mCi was incubated with 1.5×10⁷ RBL-1 cells in the presence of 20 mM serine-borate (12). In this case the yield of product with elution time 42 min under the same HPLC conditions was 1.9 nmol, and the specific activity was 24 Ci/mol from radioactivity and UV absorbance measurements (ε²⁷⁹ = 40,000). The product had absorbance maximum at 279 nm, cochromatographed with leukotriene C₃ on HPLC (Fig. 7A), and was converted by γ-glutamyl transpeptidase to a product cochromatographing with leukotriene D₃ on HPLC (Fig. 7B).³

Biological Effects of Leukotriene C₃, Leukotriene D₃, and 11-trans Leukotriene C₃ on Guinea Pig Ileum—Leukotriene C₃ and 11-trans leukotriene C₃ induced slow sustained contractions of the isolated mepyramine- and atropine-treated guinea pig ileum, with similar times of onset as observed for leukotriene C₄ (Fig. 8). The effects, which required multiple washings for reversal, were completely antagonized by a low concentration of FPL55712 (Fig. 8, cf. Ref. 20). Dose-response curves indicated that leukotriene C₃ and leukotriene C₄ were approximately equipotent and that 11-trans leukotriene C₃ was 5 to 10 times less active (Fig. 8). Leukotriene D₃ induced similar contractions as leukotriene D₄ with regard to time of onset and duration. Its potency was similar to that of leukotriene D₄ (Fig. 8).

³The tritium-labeled leukotrienes were eluted slightly before the unlabeled compounds due to isotope discrimination during HPLC (cf. Ref. 4).
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DISCUSSION

Arachidonic acid and eicosapentaenoic acid (n-3) are precursors of prostaglandins and thromboxanes (23). Recent evidence has shown that these acids are also converted to biologically active leukotrienes viz. leukotrienes C₄ and C₅ (24), respectively. Eicosatrienoic acid (n-9) which is synthesized de novo in man and other mammals, particularly when the diet is deficient in essential fatty acids (25) is not a prostaglandin or thromboxane precursor. The present results demonstrate that this acid, however, can be converted to leukotrienes of C and D types. These compounds are as potent as corresponding leukotrienes formed from arachidonic acid as determined by bioassay on guinea pig ileum. It has been postulated that some symptoms of essential fatty acid deficiency are due to decreased formation of prostaglandins. The results presented here suggest that the same may not be true for leukotrienes.

The degradative work on leukotriene C₃ has provided additional evidence for the positions of the conjugated triene and the thioether bond in leukotrienes C-E (cf. Ref. 4). The experiments with tritium-labeled 5,8,11-eicosatrienoic acid and RBL-1 cells showed that the acid was converted to leukotriene C₃ without noticeable dilution by endogenous unlabeled fatty acid. Thus, using carrier-free eicosatrienoic acid leukotrienes C₃ and D₃ of high specific activity (about 60 Ci/mmol) were biosynthesized. These compounds are suitable for investigations regarding the metabolism, excretion, and tissue distribution of leukotrienes C and D in vivo since they can be administered in tracer amounts. Such experiments are in progress.

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REFERENCES

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