Leukotriene A₄

ENZYMATIC CONVERSION INTO 5,6-DIHYDROXY-7,9,11,14-EICOSATETRAENOIC ACID BY MOUSE LIVER CYTOSOLIC EPoxide HYDROLASE

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Jesper Haeggström, Johan Meijer, and Olof Rådmark

From the Department of Physiological Chemistry, Karolinska Institutet, S-104 01 Stockholm, Sweden and the Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, S-106 91 Stockholm, Sweden

Mouse liver homogenates transformed leukotriene A₄ into a 5,6-dihydroxy-7,9,11,14-eicosatetraenoic acid. This novel enzymatic metabolite of leukotriene A₄ was characterized as a distinct enzyme as well as the compound formed in crude cytosol, but not in leukotriene B₄, a compound previously reported to be formed in liver cytosol (Haeggström, J., Rådmark, O., and Fitzpatrick, F. A. (1985) Biochim. Biophys. Acta 835, 378-384). These findings suggest a role for leukotriene A₄ as an endogenous substrate for cytosolic epoxide hydrolase, an enzyme earlier characterized by xenobiotic substrates. Furthermore, they indicate that leukotriene A₄ hydrolysis was catalyzed by a distinct enzyme among the previously described forms of epoxide hydrolases in liver.

LTA₄ is an important intermediate in the biosynthesis of leukotrienes. For review see Ref. 1. This highly unstable allylic epoxide is formed from 5-HPETE, a 5-lipoxygenase product of arachidonic acid. Two major enzymatic pathways have been described for this epoxide (Fig. 6). Hydrolysis of LTA₄, catalyzed by LTA₄ hydrolase leads to the formation of LTB₄ (2-4), while addition of glutathione by glutathione transferases leads to LTC₄ (5,6), which can be further cleaved by successive peptidases to yield leukotrienes D₅ (7,8) and E₅ (9). LTA₄ can also undergo nonenzymatic hydrolysis into the epimers at C-12 of (5S,12)-dihydroxy-6,10-trans-14-cis-eicosatetraenoic acid and two isomers of 5,6-dihydroxy-7,9,11,14-eicosatetraenoic acid (2,10). The stereochemistry of the latter two compounds has not been established.

Microsomal and cytosolic epoxide hydrolases have been described in a variety of tissues and especially in the liver from several mammalian species (11,12). Both enzymes and particularly the microsomal form have been proposed to be involved in the detoxification of potentially mutagenic and/or carcinogenic xenobiotic epoxides. Only a limited number of endogenous substrates have been reported for these enzymes.

In this paper we report the presence of a novel enzymatic pathway for LTA₄ in mouse liver, mediated by a soluble epoxide hydrolase and leading to the formation of a 5,6-dihydroxy-7,9,11,14-eicosatetraenoic acid. Recently we have also demonstrated the presence of a cytosolic LTA₄ hydrolase in human and guinea pig liver, transforming LTA₄ into LTB₄ (13). These findings suggest that epoxide hydrolase and LTA₄ hydrolysis in liver cytosol are two distinct enzymes, converting LTA₄ into 5,6-DHETE and LTB₄, respectively.

MATERIALS AND METHODS

Unlabeled LTA₄ methyl ester was from The Upjohn Co., and [14,15-3H]LTB₄ methyl ester was purchased from Amersham, United Kingdom. Saponification of the methyl ester of LTA₄ was performed in tetrahydrofurane (1 mg/µl) with argon with the addition of 1 M LiOH (0.09 µl/µg LTA) at 4 °C for 48 h. The specific activity of [14,15-3H]LTA₄ lithium salt used for incubations varied from 2.6-4.6 Ci/mol LTA₄. Synthetically prepared prostaglandin B₃ and leukotriene B₄ used as standards were from The Upjohn Co. Solid phase extractions were performed using Sep-Pak C₁₈ cartridges (Waters Associates). Methyl formate (pro analysis) and diethyl ether (ether analysis) were from Merck. HPLC stationary phases (Nucleosil C₁₈, 50-5 and 100-5, respectively) were from Macherey-Nagel, Diiren, Federal Republic of Germany, and glass-distilled HPLC solvents were purchased from Rathburn Chemicals, Walkerburn, Scotland. Pnonsacyl protectolytic enzyme (Calbiochem), diathreetol (Sigma), and soybean lipoxgynase type IV (10 µg of protein/ml, 300,000 units/mg of protein, Sigma) were all used as received.

Liver Preparations—Male or female normal fed CBA or NMRI mice (26-51 g) were anesthetized with diethyl ether and/or Mebumal Vet. (60 mg/ml, 1 mg/kg body weight). After opening the abdominal cavity, 0.5 ml of heparin (5000 IU/ml) was injected into the inferior vena cava. Via a cannula inserted into the portal vein the liver was perfused in situ with 5 ml of cold 0.9% NaCl. After cautious removal of the gallbladder, the liver was kept on ice before homogenization in 3 parts (v/w) of 50 mM potassium phosphate buffer, pH 7.4, using a Potter-Elvehjem homogenizer with a glass or Teflon pestle. Homogenates were centrifuged at 20,000 x g for 30 min. The supernatant was incubated with leukotriene A₄ methyl ester at 37 °C for 48 h.
was recentlyrifued at 105,000 × g for 60 min. (The resulting high speed supernatant is referred to below as liver cytosol.) Pellets were washed and recentrifuged once and then resuspended in a defined volume of buffer. All centrifugations were performed at 4°C.

**Purification of Mouse Liver Cytosolic Epoxide Hydrolase—**Cytosolic epoxide hydrolase was purified to apparent homogeneity from the liver of untreated and clofibrate-treated male mice. The purification procedure involved column chromatography on DEAE-cellulose, phenyl-Sepharose, and hydroxyapatite as previously described (14). In most of our experiments enzyme purified from clofibrate-treated mice was used, and the results did not differ from those obtained with enzyme from untreated mice.

**Incubations and Extractions—**Aliquots of 500 μl of different liver subcellular fractions were incubated at 37°C for 10 min with [3H]LTA₄, lithium salt (20 μM) added as an ethanol solution. Reactions were stopped with 4 volumes of methanol containing a defined amount of prostaglandin B₂ or B₃ (internal standard). Precipitated proteins were removed by filtration and washed with methanol. The combined filtrates were evaporated under reduced pressure, reconstituted in 1 ml of methanol, and diluted with 9 ml of water. After acidification to pH 3 with 1 M HCl, LTA₄ transformation products were extracted on a Sep-Pak C₁₈ cartridge. Adsorbed products were purified by consecutive washing with 10 ml of redistilled water and 10 ml of hexane and finally eluted with 10 ml of methyl formate (15). Approximately 85% of adsorbed radioactivity was recovered in the last fraction. The methyl formate fraction was evaporated under a stream of N₂, and the residue was reconstituted in HPLC mobile phase and analyzed by RP-HPLC.

Similar incubations were performed with purified cytosolic epoxide hydrolase in 0.1 M Tris-HCl buffer, pH 8. The buffer contained 0.1 mM dithiothreitol and was saturated with argon to reduce oxidation of the enzyme, thereby optimizing its activity (17). In some experiments with purified cytosolic epoxide hydrolase, LTA₄-derived products were isolated by acidic diethyl ether extractions.

**Instrumental Analysis—**For RP-HPLC a column (250 × 4.5 mm) packed with Nucleosil C₁₈ was eluted with methanol/water/acetic acid, 70:25:0.01, v/v, at 1 ml/min. For SP-HPLC a column (250 × 4.5 mm) packed with Nucleosil 100-5 or 50-5, eluted with hexane/2-propanol/acetate acid, 97.5:2:50:2.0, v/v, at 1 or 1.5 ml/min, was used. The HPLC eluate was collected in discrete chromatographic fractions of 2.5 or 1 ml each and subjected to β-scintillation counting.

Quantitations of LTA₄ metabolites were based on peak height ratios between the internal standard (PGB₁/PGB₂) and the respective compound (19) which also compensated for losses during the various steps of purification. Alternatively, compound D (5,6-DHETE) was quantitated by peak height measurements from standard injections of known amounts of compound. The concentration of 5,6-DHETE in the standard solution was calculated by ultraviolet spectroscopy using an extinction coefficient of 4.0 × 10⁴ M⁻¹ cm⁻¹. Ultraviolet spectra were recorded on a Hewlett-Packard 8450 A spectrophotometer with methanol as solvent.

For gas chromatograph-mass spectrometry (GC/MS) a Dani 3800 gas chromatograph was connected to a VG 7070E mass spectrometer. The capillary column (fused silica, 25 m × 0.33 mm (inner diameter), OV-1, film thickness 0.15 μm, Scandinaviska Genetec, Malmö, Sweden) was operated isothermally at temperatures between 240–250°C with helium as carrier gas. The temperature of the ion source was maintained at 250°C, and the energy of the ionization beam was 22.5 eV. For GC/MS, samples were converted to the methyl esters, trimethylsilyl ethers, by treatment with diazomethane in diethyl ether. The GC/MS instrument was operated isothermally at temperatures between 240–250°C with helium as carrier gas. The temperature of the ion source was maintained at 250°C, and the energy of the ionization beam was 22.5 eV. For GC/MS, samples were converted to the methyl esters, trimethylsilyl ethers, by treatment with diazomethane in diethyl ether. The GC/MS instrument was operated isothermally at temperatures between 240–250°C with helium as carrier gas. The temperature of the ion source was maintained at 250°C, and the energy of the ionization beam was 22.5 eV. For GC/MS, samples were converted to the methyl esters, trimethylsilyl ethers, by treatment with diazomethane in diethyl ether. The GC/MS instrument was operated isothermally at temperatures between 240–250°C with helium as carrier gas.

**RESULTS**

**Incubations of Mouse Liver with LTA₄—**When mouse liver homogenate or cytosol was incubated with [3H]LTA₄, RP-HPLC detected two major (C and D) and three minor (A, B, and E) ultraviolet-absorbing radioactive peaks (Fig. 1). Peaks A and B cochromatographed with standard compounds (Δ⁴-trans-LTB₄ and 12-epi-Δ⁴-trans-LTB₄, respectively) formed by nonenzymatic hydrolysis of LTA₄ (2). Peak C cochromatographed with a leukotriene B₃ standard, demonstrating the presence of a soluble LTA₄ hydrolase activity in mouse liver. This finding confirms previous data reported for human and guinea pig liver incubated with LTA₄ (13). The major peak D which eluted late in RP-HPLC and exhibited an ultraviolet spectrum with triplet peaks at 263, 272, and 284 nm was subjected to further structural analysis (see "Structure of Compound D"). The minor peak E (doubtful peak) was found to be a mixture of several compounds, as judged by SP-HPLC analysis of the methyl ester derivatives. No further identification of these compounds was performed.

In control incubations with buffer alone the product pattern was dominated by peaks A and B, the two major nonenzymatic hydrolysis products of LTA₄ (Fig. 1). As expected, peak C (LTB₃) was practically absent and only a minor peak D could be detected, indicating that its formation in liver was attributable to an enzymatic activity.

Mouse liver homogenate was subjected to differential centrifugation and the subcellular fractions (20,000 × g pellet, 105,000 × g pellet, and 105,000 × g supernatant) were incubated with LTA₄ (20 μM). The result from one typical experiment is shown in Table I. Formation of compound D was...
TABLE I
Formation of compound D and LTB4 in subcellular fractions of mouse liver

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Compound D</th>
<th>LTB4</th>
</tr>
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<tbody>
<tr>
<td>Homogenate</td>
<td>12</td>
<td>7</td>
</tr>
<tr>
<td>20,000 x g pellet</td>
<td>13</td>
<td>&lt;1</td>
</tr>
<tr>
<td>105,000 x g pellet</td>
<td>1</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>105,000 x g supernatant</td>
<td>13</td>
<td>5</td>
</tr>
</tbody>
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Amount of compound formed in each subcellular fraction is expressed as nmol recovered per g of liver tissue. The result represents one typical experiment.

Enzymatic Transformation of LTA4 into 5,6-DHETE

Mouse liver was homogenized in 50 mM potassium phosphate buffer, pH 7.4 (3 ml/g). The homogenate was subjected to differential centrifugation to obtain the subcellular fractions (abulated: 500 µl of each fraction was incubated with 20 µM LTA4 (37 °C, 10 min), and products were analyzed by RP-HPLC. Formation of LTB4 and compound D was calculated as described under “Materials and Methods.” Amount of compound formed in each subcellular fraction is expressed as nmol recovered per g of liver tissue. The result represents one typical experiment.

In contrast to these findings with cytosolic epoxide hydrolase from mouse a purified microsomal epoxide hydrolase from predominantly seen in the 20,000 x g pellet and the 105,000 x g supernatant while formation of leukotriene B4 was confined to the high speed supernatant. At a substrate concentration of 20 µM, compound D was in fact the most dominant transformation product of LTA4 in mouse liver cytosol. Under these conditions compound D accounted for approximately 25% of added substrate while approximately 10% was converted into leukotriene B4. Values in the left column of Table I are not additive, probably due to suboptimal substrate concentrations.

To ascertain the enzymatic nature of the transformation of LTA4 into compound D, aliquots of mouse liver cytosol was heated at 80 °C for 10 min or treated with a proteolytic enzyme (Pronase) for 3 h at 37 °C and incubated with [3H]LTA4. Both regimens almost completely inhibited the formation of compound D, as seen in Fig. 2. However, when cytosol was heated to 66 °C for 1 h and incubated with LTA4, no formation of LTB4 could be seen while formation of compound D was clearly detected but reduced to approximately one-third of the control value.

In addition, liver cytosol from another species, guinea pig, was also found to convert leukotriene A4 into compound D. For structural analysis of this material, see “Structure of Compound D.”

Incubation of Purified Epoxide Hydrolase with LTA4—A purified cytosolic epoxide hydrolase of mouse liver origin was incubated with 20 µM [3H]LTA4. When products were analyzed by RP-HPLC, four ultraviolet absorbing radioactive peaks appeared (A, B, D, and E) (Fig. 1). Upon comparison of the chromatographic profile to the one obtained from incubations of mouse liver cytosol, the only difference seen was the lack of an ultraviolet-absorbing radioactive peak C. Thus we could conclude that no leukotriene B4 was formed from LTA4 by cytosolic epoxide hydrolase (Fig. 1). However, compound D was an abundant product also in these incubations.

When various amounts of purified cytosolic epoxide hydrolase (0-16 X 10^-5 mg/ml) was incubated at a constant concentration of LTA4 (60 µM) and the amount of product (compound D) formed was plotted as a function of enzyme concentration, a linear relationship could be seen (correlation coefficient > 0.99) (Fig. 3). Finally, heat treatment of the purified enzyme (80 °C, 10 min) almost completely abolished the formation of compound D, indicating its enzymatic nature (Fig. 3).

In contrast to these findings with cytosolic epoxide hydrolase from mouse a purified microsomal epoxide hydrolase from rat liver (14) did not metabolize LTA4. In such incubations no enzymatic formation of LTB4 or compound D was detectable.

Compound D was thus obtained from incubations of both crude cytosol and a purified cytosolic epoxide hydrolase of mouse liver origin with LTA4. The structural data presented below was determined using material derived from the purified enzyme.

Structure of Compound D—Material collected under peak D (RP-HPLC) exhibited an ultraviolet spectrum with triplet maxima at λmax = 263, 272, and 284 nm, demonstrating the presence of a conjugated triene moiety (Fig. 4, panel B). GC/MS analysis of this material (methyl esters, trimethylsilyl ethers) revealed two peaks (D-I, D-II) with C values of 23.8 and 24.7, respectively (OV-1). Both compounds exhibited similar mass spectra with ions of high intensity at m/e 99, 113 (203-90), 125, 147 (Me2SiO+-SiMe3), 171 (203-32), 203 (Me2SiO+-CH2(CH2)5-COOCH3), 225, 291 loss of -CH (OSiMe3)-(CH2)5-COOCH3, 393 (M-101, loss of -CH2(CH2)5-COOCH3), 404 (M-90), 463 (M-31), and 479 (M-15), compatible with tetraunsaturated C20 fatty acids carrying hydroxyl groups at C-5 and C-6. In Fig. 4, panel C, the mass spectrum of isomer D-I (C=24.7) is shown. The spectrum of isomer D-I differed only in the intensities of some ions (225-479) and was in good agreement with previously published data for 5,6-DHETE (2). The relative amounts of

![Fig. 2. RP-HPLC analysis of products formed when heat-treated (80 °C, 10 min) and untreated (control) mouse liver cytosol (10 mg of protein/ml) was incubated with 20 µM [3H]LTA4 (37 °C, 10 min). The column (Nucleosil C18, 250 × 4.5 mm) was eluted with methanol/water/acetic acid, 70:30:0.01, v/v, at 1 ml/min. Upper panels show the distribution of tritium in each collected fraction as determined by liquid scintillation counting. Lower panels show the continuous recordings of ultraviolet absorption at 270 nm. Vertical lines indicate the alignment of radioactivity and absorbance.](image-url)
Enzymatic Transformation of LTA₄ into 5,6-DHETE

FIG. 3. Formation of compound D at various concentrations of cytosolic epoxide hydrolase. Samples with various concentrations of purified cytosolic epoxide hydrolase from mouse liver (0-160 μg/ml) were incubated at a constant concentration of LTA₄ (60 μM, 37 °C, 1 min). The enzyme was dissolved in argon-saturated 0.1 M Tris-HCl buffer, pH 8, with the addition of 0.1 mM dithiothreitol. Amounts of product formed (compound D) was estimated by RP-HPLC. The column (Nucleosil CIS, 250 × 4.5 mm) was eluted with methanol/water/acetic acid, 75:25:0.01, v/v, at 1 ml/min. Quantitations were based on measurements of the peak height ratio between PGB (internal standard) and compound D. A square (C3) denotes the result obtained with heat-treated (90 °C, 10 min) enzyme (160 μg/ml).

FIG. 4. Analytical data for compound D formed in incubations of purified cytosolic epoxide hydrolase with LTA₄. Panel A, SP-HPLC analysis (Nucleosil 50-5, 200 × 4.5 mm, hexane/2-propanol/acetic acid, 97.5:2.5:0.2, v/v, at 1.5 ml/min) of the methyl ester derivative of material collected under peak D from RP-HPLC. Solid tracing depicts the ultraviolet absorbance at 270 nm and dotted tracing the distribution of tritium in each collected fraction as determined by liquid scintillation counting. Panel B, ultraviolet spectrum of compound D in methanol. Panel C, Mass spectrum of compound D (isomer D-II, C-value 24.7).

these two isomers varied between different samples with a clear predominance (>70%) for isomer D-II (C-24.7). However, when material collected under peak D (RP-HPLC) was subjected to SP-HPLC analysis (methyl ester), only one apparently homogenous peak appeared (Fig. 4, panel A). It is thus uncertain whether the GC/MS analysis detected two separate compounds present under peak D or reflected an isomerization during the purification and analysis of one initially formed compound. When the material under peak D was subjected to catalytic hydrogenation and analyzed by GC/MS a single peak appeared with a C-value of 23.6. Prominent ions in the mass spectrum were observed at m/e 129, 147, 171 (203-32), 203 (base peak, Me₂SiO≡CH—(CH₂)₃—COOCH₃), 276 (CH(OSiMe₃)—(CH₂)₃—C(OCH₃)—O=SiMe₃) from a rearrangement, 299 (loss of CH(OSiMe₃)—(CH₂)₃—COOCH₃, 336, 337, 393, 471 (M-31), and 487 (M-15). This mass spectrum was in accordance with earlier published data for a dihydroxylated C₂₀ saturated fatty acid with the hydroxyl groups at C-5 and C-6 (2). Based on these data compound D was assigned the covalent structure 5,6-dihydroxy-7,9,11,14-eicosatetraenoic acid.

In order to obtain additional information about the location and geometry of double bonds, a sample of compound D was subjected to soybean lipoxygenase conversion. Thus, 3.2 μg of compound D was incubated with soybean lipoxygenase (4 μg, 300 units/μg) in 1 ml of 0.1 M sodium borate buffer, pH 8.5, at 0 °C. The reaction was followed by consecutive recordings of the ultraviolet spectrum (220–400 nm) at 5-min intervals. As shown in Fig. 5, the triplet spectrum of 5,6-DHETE with maximum at 274 nm (in buffer) was completely shifted within 30 min to another triplet spectrum with maximum at 302 nm (in buffer), typical of a conjugated tetranea moiety and in accordance with the formation of a 5,6-dihydroxy-15-hydroperoxy-7,9,11,13-eicosatetraenoic acid. Soybean lipoxygenase catalyzes the transformation of an unsaturated fatty acid into the corresponding hydroperoxy fatty acid only if a cis,cis-1,4-pentadiene structure with a methylene unit at C-3 is present in the substrate (18). Therefore, we could conclude that the Δ⁵ and Δ¹⁴ double bonds in 5,6-DHETE both had cis configurations.

Practically identical analytical data were obtained for compound D isolated from incubations of mouse liver cytosol. GC/MS analysis of hydrogenated compound and soybean lipoxgenase conversion were carried out only with material generated by purified cytosolic epoxide hydrolase.

Structural analysis of compound D obtained from incubations of guinea pig liver cytosol was based on RP-HPLC, ultraviolet spectroscopy, and GC/MS and revealed analogous data as compared to material derived from mouse liver cytosol.
**DISCUSSION**

In this paper we describe the formation of 5,6-DHETE in mouse liver incubated with leukotriene A₄. The structural elucidation was based on chromatographic characteristics, ultraviolet spectroscopy, GC/MS, and the enzymatic conversion of the compound by soybean lipoxigenase. Heat sensitivity and susceptibility to proteolytic digestion support the conclusion that this transformation of LTA₄ was attributable to an enzymatic activity. In mouse liver cytosol incubated with LTA₄, formation of LTB₄ was also prominent, as previously reported for guinea pig and human liver (13).

When cytosolic epoxide hydrolase purified to apparent homogeneity from mouse liver was incubated with LTA₄, 5,6-DHETE was also formed, with physicochemical characteristics indistinguishable from the compound formed in mouse liver cytosol. However, cytosolic epoxide hydrolase did not transform LTA₄ into LTB₄. These data indicate that LTA₄ may be hydrolyzed enzymatically in two ways by two separate enzymes. Thus, LTA₄ hydrolase and epoxide hydrolase catalyze the formation of leukotriene B₄ and 5,6-DHETE, respectively, from LTA₄ in liver cytosol. This concept is further supported by the different heat sensitivities of the respective enzyme. Treatment at 56 °C for 1 h of mouse liver cytosol completely abolished the LTA₄ hydrolase activity while cytosolic epoxide hydrolase activity with regard to inhibition, molecular weight, and substrate selectivity (16). Also of interest in this context are some physical properties of LTA₄, hydrolase, recently purified to apparent homogeneity from human neutrophils (19). The monomeric molecular weight and quaternary structure of this enzyme were found to be different from the properties reported for a purified cytosolic epoxide hydrolase from human liver (20). Furthermore, the leukocyte enzyme did not catalyze the hydrolysis of trans- or cis-stilbene oxide into the corresponding vicinal diols. These two epoxides are well characterized substrates for different forms of epoxide hydrolase (17).

The enzymatic hydrolysis of LTA₄, catalyzed by cytosolic epoxide hydrolase and leading to the formation of 5,6-DHETE, represents a novel enzymatic pathway in the leukotriene cascade (Fig. 6). Two diastereoisomers of 5,6-DHETE can also be formed from LTA₄ by nonenzymatic hydrolysis (2). 5,6-DHETE formed from LTA₄ in crude cytosol or preparations of purified cytosolic epoxide hydrolase appeared as one compound in two HPLC systems. However, GC/MS analysis revealed two isomers (D-I and D-II). The major component (D-II) had a C-value of 24.7 in contrast to the C-value of D-I (23.8) and previously published values (C-23.8 and C-23.9) for the two nonenzymatic diastereoisomers (2). GC/MS analysis of hydrogenated material revealed only one peak, suggesting that the two components detected before hydrogenation (D-I and D-II) are not diastereoisomers but rather geometrical isomers. These observations could be explained by partial isomerization of enzymatically formed 5,6-DHETE during the analytical procedure or by contamination of the enzymatic product by nonenzymatically formed 5,6-DHETE, separable by GC but not by HPLC.

Leukotriene A₄ is a trans epoxide with three conjugated double bonds. Since mouse liver cytosolic epoxide hydrolase catalyzes the opening of a trans epoxide stereospecifically in a trans manner to give the corresponding *erythro*-diol (21), it may be assumed that the stereochemistry of the vicinal diol in 5,6-DHETE is in *erythro* configuration. Because of the proximity to the Δ² double bond, C-6 should be more susceptible than C-5 to a nucleophilic attack of water, and the orientation of the hydroxyl group at C-5 will, therefore, be retained from LTA₄. Thus the most probable configuration of the hydroxyl groups in 5,6-DHETE formed via enzymatic hydrolysis of LTA₄ will be (5S,6R).

Regarding the double bond geometry of 5,6-DHETE, some features may be assumed. During the opening of the oxirane ring the three conjugated double bonds at Δ¹,Δ³, and Δ¹⁴ will probably remain intact, provided that the enzymatic reaction does not involve migration of charge or a radical in the conjugated triene of the substrate. Furthermore, when 5,6-

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*J. Haeggström, J. Meijer, and O. Rådmark, unpublished data.*
DHETE was subjected to soybean lipoxygenase digestion, a shift in the ultraviolet spectrum was recorded with the appearance of a spectrum typical of a conjugated tetraene, indicating the formation of a 5,6-dihydroxy-15-hydroperoxy-7,9,11,13-eicosatetraenoic acid (Fig. 5). The same type of reaction has previously been reported, using LTC₄ as substrate (5). Soybean lipoxygenase acts on a cis,cis-1,4-penta diene structure, suggesting that the Δ¹¹ and Δ¹⁴ double bonds of 5,6-DHETE are both in a cis configuration. A tentative structure of 5,6-DHETE formed enzymatically from LTA₄ thus would be: (5S,6R)-dihydroxy-7,9-trans-11,14-cis-eicosatetraenoic acid.

Cytosolic epoxide hydrolase is a widely distributed enzyme, but so far only a limited number of endogenous substrates have been reported (22). Additional studies are required in order to elucidate whether LTA₄ is a substrate for cytosolic epoxide hydrolase also in vivo, generating significant amounts of 5,6-DHETE in various tissues.

At present, work is in progress in our laboratory to establish the biological significance of this novel enzymatic metabolite of leukotriene A₄.

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REFERENCES