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Biochemistry

Enzyme with dual lipoxygenase activities catalyzes leukotriene A_4 synthesis from arachidonic acid

(potato lipoxygenase/bishomo-γ-linolenic acid/8-lipoxygenase/*D*-hydrogen/5-hydroperoxyicosatetraenoic acid)

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ABSTRACT **When arachidonic acid was incubated with homogenates of potato tubers, two isomers of 6-*trans*-leukotriene B_4, epimeric at C-12, were formed in addition to the major product, (5*S*-hydroperoxy-6-*trans*-8,11,14-*cis*-icosatetraenoic acid (5-HPETE). To elucidate the mechanism of biosynthesis of the dihydroxy-acids, the lipoxygenase from the potato tubers was purified to apparent homogeneity by a combination of conventional chromatographic procedures and high-performance liquid chromatography equipped with a chromatofocusing column (Mono-P). The purified lipoxygenase acted on arachidonic acid and bishomo-γ-linolenic acid to yield (5*S*)-hydroperoxy- and (8*S*)-hydroperoxyicosanoids, respectively. Furthermore, the purified enzyme converted 5-HPETE to leukotriene A_4, with the presence of the epoxide intermediate being demonstrated by $^{18}O_2$ experiments, methanol trapping, as well as further conversion to leukotriene B_4 by the purified leukotriene A_4 hydrolase. Several experiments, including those with lipoxygenase inhibitors, heat treatment, and competitive inhibition, indicated that both the 5-lipoxygenase and leukotriene A_4 synthase activities resided in the same protein and that the formation of leukotriene A_4 from 5-HPETE was catalyzed by the 8-lipoxygenase activity of the enzyme.**

Leukotrienes (LT) are potent bioactive compounds involved in inflammation, allergy, and several deteriorative disorders (1). The biosynthesis of LT is initiated by the 5-lipoxygenation of arachidonic acid to produce (5*S*)-hydroperoxy-6-*trans*-8,11,14-*cis*-icosatetraenoic acid (5-HPETE), which is further transformed to (5*S*)-*trans*-5,6-oxido-7,9-*trans*-11,14-*cis*-icosatetraenoic acid (LTA_4), a key intermediate in the formation of LT (2–12). Alternatively, 5-HPETE is decomposed to (5*S*)-hydroxy-6,8,11,14-icosatetraenoic acid (5-HETE), a relatively inactive compound, by either peroxidase or a nonenzymatic reaction. Therefore, it is of primary importance to characterize LTA_4 biosynthesis for the understanding of the regulatory mechanism of LT synthesis. Because experiments using double-radiolabeled arachidonate demonstrated the stereospecific elimination of a hydrogen (10 *D*, pro-*R*) at C-10 as the initial step to form an allylic epoxide (11, 12), it was reasonable to consider the involvement of lipoxygenase in this reaction. The present study, using a homogeneous preparation of potato lipoxygenase, demonstrated clearly that the 8-lipoxygenase activity of the enzyme may catalyze the formation of LTA_4 from 5-HPETE.

EXPERIMENTAL PROCEDURES

Materials. Commercial sources of reagents, enzymes, and materials were as follows: [1-^{14}C]arachidonic acid (55.5 mCi/mmol; 1 Ci = 37 GBq) and [1-^{14}C]bishomo-γ-linolenic acid (53 mCi/mmol), from the Radiochemical Centre, Amersham, and New England Nuclear, Boston, respectively; oxygen-18 gas (99.5% pure), from Ventron, GmbH; arachidonic acid, from Nu Check Prep (Elysian, MN); and bishomo-γ-linolenic acid and linoleic acid, from Sigma. All fatty acids were used after purification by silicic acid chromatography. Phenyl disulfide was from Fluka (Buchs, Switzerland); DEAE-Sephadex A-50, Sephacryl S-300, and the fast protein liquid chromatography (FPLC) system with a Mono-P HR(5/20) column and Polybuffer 74 were from Pharmacia. 5,8,11-Icosatriynoic acid was prepared by the method described previously (13, 14). Prostaglandin B_2 (PGB_2) and 5,6-methano-LTA_4 were donated from Ono Pharmaceutical (Osaka, Japan). BW755 C (3-amino-1-(3-trifluoromethylphenyl)-2-pyrazoline·HCl) was supplied by the Wellcome Research Laboratories (Beckenham, England). Hydroxyapatite was prepared by the method of Tiselius *et al.* (15). LT [(5*S*,12*R*)-dihydroxy-6-*cis*-8,10-*trans*-14-*cis*-icosatetraenoic acid (LTB_4), 6-*trans*-LTB_4, and 12-*epi*-6-*trans*-LTB_4] and 5-HPETE were biosynthesized as described (3, 16). LTA_4 was synthesized from 5-HPETE (17). LTA_4 hydrolase was purified from human leukocytes (unpublished data).

Assay of Lipoxygenase. The lipoxygenase activity was measured either by a spectrophotometer (Cary model 219) or an oxygen monitor (YSI model 53, Yellow Springs Instrument) in the standard assay mixture consisting of 0.1 M potassium phosphate buffer at pH 6.3, enzyme, and 100 μM linoleic acid. One unit of enzyme activity was defined as the amount of enzyme that produced 1 μmol of product (or that consumed 1 μmol of oxygen) per min at 24°C. The specific activity was expressed as the number of units per mg of protein. The protein concentration was determined according to the method of Lowry *et al.* (18) using bovine serum albumin as standard. The activities toward [1-^{14}C]arachidonic acid and [1-^{14}C]-bishomo-γ-linolenic acid were also measured by TLC as described (8).

Assay of LTA_4 Synthase. The reaction mixture contained 0.1 M potassium phosphate buffer at pH 6.3, 100 μM 5-HPETE (100 nmol dissolved in 10 μl of ethanol), and enzyme in a total volume of 1 ml. Incubation was started by the addition of substrate and was carried out at 24°C for 10 min. The reaction was terminated by acidification to pH 3 (1 M HCl), and the hydrolysis products of LTA_4 were extracted and purified as described (3). After treatment with ethereal diazo-

Abbreviations: LT, leukotriene(s); 5-HPETE, (5*S*)-hydroperoxy-6-*trans*-8,11,14-*cis*-icosatetraenoic acid; 5-HETE, (5*S*)-hydroxy-6,8,11,14-icosatetraenoic acid; 11-HPETE, (11*S*)-hydroperoxy-5,8-*cis*-12-*trans*-14-*cis*-icosatetraenoic acid; 5,12-diHETE, (5*S*,12*S*)-dihydroxy-6-*trans*-8-*cis*-10-*trans*-14-*cis*-icosatetraenoic acid; LTA_4, (5*S*)-*trans*-5,6-oxido-7,9-*trans*-11,14-*cis*-icosatetraenoic acid; LTB_4, (5*S*,12*R*)-dihydroxy-6-*cis*-8,10-*trans*-14-*cis*-icosatetraenoic acid; 5,12-diHPETE, (5*S*,12*S*)-dihydroperoxy-6-*trans*-8-*cis*-10-*trans*-14-*cis*-icosatetraenoic acid; RP-HPLC, reversed phase HPLC; SP-HPLC, straight-phase HPLC; PG, prostaglandin; GC/MS, gas chromatography/mass spectrometry; FPLC, fast protein liquid chromatography.

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methane, the samples were analyzed by reversed phase HPLC (RP-HPLC) (Nucleosil C-18, 250 × 4.6 mm; methanol/H_2O/acetic acid, 75:25:0.01; flow rate, 1 ml/min) or straight-phase HPLC (SP-HPLC) (Nucleosil 50-5, 250 × 4.6 mm; hexane/2-propanol/acetic acid, 96:4:0.01; flow rate, 1 ml/min). Because >80% of the products of nonenzymatic hydrolysis of LTA_4 were 6-*trans*-LTB_4 and its 12-epimer (3), the enzyme activity was determined by measuring the ratio of peak heights of these two compounds to that of PGB_2 (0.1–1 μg) added as an internal standard. All HPLC was carried out with a LDC constametric pump model III equipped with a Spectro monitor III model 1204A set at 270 nm.

Purification of Lipoxygenase from Potato Tubers. Homogenization, ammonium sulfate fractionation (25–50%), and chromatographies on hydroxyapatite and DEAE-Sephadex A-50 were performed according to the method of Sekiya *et al.* (19). After DEAE-Sephadex A-50 (4 × 50 cm) chromatography, the sample was applied to a Sephacryl S-300 column (3 × 80 cm), which was equilibrated with 20 mM Tris·HCl buffer at pH 7.2 containing 0.1 M NaCl. Elution was carried out with the same buffer at a flow rate of 0.6 ml/min. The enzyme activity eluted at around 300 ml. The active fraction (30 ml) was combined and applied to a second DEAE-Sephadex A-50 column (2 × 10 cm), which was equilibrated with 20 mM Tris·HCl buffer at pH 7.2 containing 0.1 M NaCl. A linear gradient elution was carried out with 150 ml each of 20 mM Tris·HCl buffer at pH 7.2 containing 0.1 M NaCl and the same buffer containing 0.5 M NaCl. The active fraction was concentrated to about 2 ml by using a PM-10 diaflo membrane, dialyzed for 1 hr against 100 vol of 25 mM piperazine·HCl buffer at pH 5.3, and injected onto a FPLC Mono-P column that was also equilibrated with the same piperazine buffer (operated at room temperature). The enzyme was eluted with 10% Polybuffer 74·HCl at pH 4 at a flow rate of 0.75 ml/min (pressure, 2.2 MPa) and appeared at around pH 4.5 (Fig. 2). The pH of the enzyme solution was adjusted to pH 6.2 by the addition of 1 M K_2HPO_4. The enzyme was kept at −70°C for 1 wk without any appreciable loss of the activity. In some cases, Polybuffer 74 was removed by precipitating the enzyme with 80% saturated ammonium sulfate for 2 hr, followed by dialysis against 20 mM potassium phosphate buffer at pH 6.2.

Miscellaneous. Labeling experiments with $^{18}O_2$ and trapping of LTA_4 with methanol were performed essentially as described (4), except that a purified enzyme preparation (2.2 mg, 15.2 units/mg of protein) was used instead of cells. Oxidative ozonolysis and steric analysis were carried out as described (2, 20, 21). Menthylchloroformate derivatives of dimethyl 2-DL-hydroxyazelaic acid were kindly donated by M. Hamberg of this department.

Gas chromatography/mass spectroscopy (GC/MS) was performed as described (4). The molecular weight of the enzyme was calculated according to the method of Laurent and Killander (22) by using a Sephacryl S-300 column (1.8 × 75 cm), which was equilibrated with 50 mM potassium phosphate buffer at pH 6.8 containing 0.1 M NaCl. Marker proteins were bovine liver catalase (M_r 240,000), bovine liver lactate dehydrogenase (M_r 120,000), bovine serum albumin (M_r 67,000), and soybean trypsin inhibitor (M_r 20,500).

$NaDodSO_4$/polyacrylamide gel electrophoresis was carried out by the method of Laemmli (23) with a marker protein kit (MW-SDS-200) supplied by Sigma.

RESULTS

Transformation of Arachidonic Acid with Extracts of Potato Tubers. When arachidonic acid was incubated with the ammonium sulfate fraction of the potato homogenate, 5-HPETE was the major product. The compound was reduced with $NaBH_4$ and its identity with 5-HETE was confirmed by GC/MS (data not shown). A small amount of (11*S*)-hydroperoxy-5,8-*cis*-12-*trans*-14-*cis*-icosatetraenoic acid (11-HPETE) (about 5% of 5-HPETE) was also synthesized. When the ethyl acetate fraction of a CC-4 column was analyzed by SP-HPLC, at least three major compounds (peaks I, II, and III in Fig. 1) were observed, all of which showed characteristic LT UV spectra. When the sample was reduced with $NaBH_4$ prior to SP-HPLC, peak I shifted to peak I′, which had the same retention time as (5*S*,12*S*)-dihydroxy-6-*trans*-8-*cis*-10-*trans*-14-*cis*-icosatetraenoic acid (5,12-diHETE). The structure of the reduced compound was identified as 5,12-diHETE by both UV absorption and GC/MS (C value of 22.4 with 1% SE-30; mass spectrum (*m/e*): 404 (M−90), 354, 293, 279, 213, 203 (base peak), 171, and 129). Therefore, compound I was tentatively identified as (5*S*,12*S*)-dihydroperoxy-6-*trans*-8-*cis*-10-*trans*-14-*cis*-icosa-tetraenoic acid (5,12-diHPETE). Peaks II and III were identified as 6-*trans*-LTB_4 and 12-*epi*-6-*trans*-LTB_4, respectively, according to the retention times on SP-HPLC, UV absorbances, and C values (24.8, 1% SE-30), as well as their mass spectra that exhibited major ions at (*m/e*): 404 (M−90), 383, 293, 267, 217, 203, and 129 (base peak). LTB_4 could not be detected. To elucidate the mechanism of the biosynthesis of these three compounds (5,12-diHPETE, 6-*trans*-LTB_4, and 12-*epi*-6-*trans*-LTB_4), the potato lipoxygenase was purified to homogeneity.

Purification and Molecular Properties of Potato Lipoxygenase. The purification was carried out by the method described under *Experimental Procedures*. FPLC with Mono-P column was used (Fig. 2) instead of preparative polyacrylamide gel electrophoresis employed by Sekiya *et al.* (19). This resulted in relatively higher recovery (14% as compared to 3%) and higher specific activity (43 as compared to 25 units/mg of protein). The constant ratio (4.4–6.8) of the lipoxygenase activities toward two fatty acids (linoleic acid and arachidonic acid) through the purification suggested that a single enzyme acted on both substrates. The purified enzyme was apparently homogeneous, as judged by $NaDodSO_4$/polyacrylamide gel electrophoresis (Fig. 2 *Inset*) and gel filtration on Sephacryl S-300 (Fig. 3). The M_r was determined as 96,000 by gel filtration and as 94,000 by $NaDodSO_4$/polyacrylamide gel electrophoresis either in the presence or absence of 1 mM dithiothreitol, indicating that the enzyme was a monomeric protein of M_r 95,000 ± 1,000. The isoelectric point (pI) was 4.5.

Reaction of the Purified Enzyme with Arachidonic Acid, Bishomo-γ-linolenic Acid, and 5-HPETE. Arachidonic acid was converted to 5-HPETE at the rate of 7.6 μmol/min per mg at 24°C by the purified enzyme, whereas bishomo-γ-linolenic

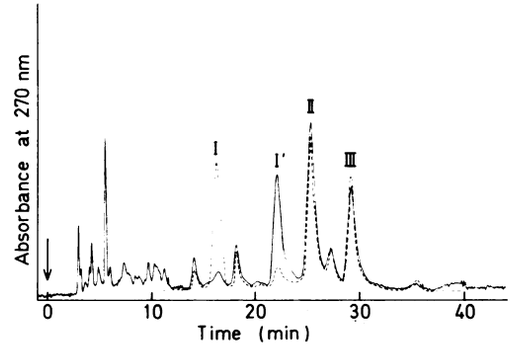

FIG. 1. SP-HPLC of the reaction products obtained by the incubation of arachidonic acid (50 mg) with the ammonium sulfate fraction (1.240 mg of protein) of potato tubers. The ethyl acetate fraction of CC-4 column chromatography was treated with ethereal diazomethane and the aliquot was applied to a column (250 × 4 mm) both before (---) and after (—) $NaBH_4$ treatment. The solvent used was hexane/2-propanol/acetic acid (96:4:0.01; flow rate, 1 ml/min).

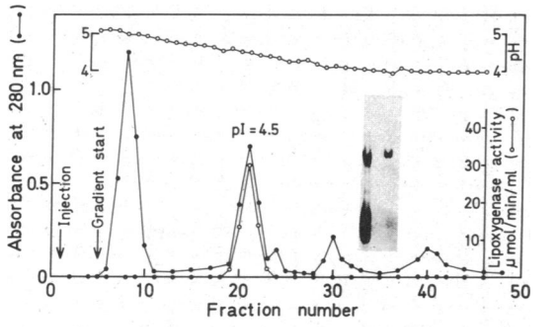

FIG. 2. Purification of potato lipoxygenase by FPLC equipped with a Mono-P HR column (200 × 5 mm). The column was equilibrated with 25 mM piperazine·HCl buffer at pH 5.3. Elution was carried out with 40 ml of 10% Polybuffer 74·HCl at pH 4 at a flow rate of 0.75 ml/min. It was carried out at room temperature. The eluate was collected in 1-ml fractions and pH (○), absorbance at 280 nm (●), and the lipoxygenase activity (○) were measured. (*Inset*) $NaDodSO_4$/polyacrylamide gel electrophoresis (7.5% polyacrylamide/0.1% $NaDodSO_4$): left lane, samples before FPLC (≈25 μg); right lane, about 10 μg of the purified lipoxygenase.

acid gave rise to 8-hydroperoxyicosatrienoic acid (70–80%) at the rate of 3.2 μmol/min per mg with two minor products (11-hydroperoxy and 12-hydroperoxy acids). These three hydroperoxy acids were reduced to 8-, 11-, and 12-hydroxy derivatives, which were structurally confirmed by GC/MS (data not shown). About 25 μg of the methyl ester of 8-hydroxy acid was derivatized with menthylchloroformate and was subjected to oxidative ozonolysis (2, 20, 21). Gas chromatography (3% OV-210) gave the peak corresponding to the menthoxycarbonyl derivative of dimethyl-2-L-hydroxyazelate (95%). The result indicates that the compound had the D-configuration at C-8, as in the case of the leukocyte product (2).

When the purified enzyme was incubated with 100 μM 5-HPETE for 10 min at 24°C, 5,12-diHPETE and two isomers of 6-*trans*-LTB_4 were formed, as observed with the reaction using crude extracts. The ratio of 5,12-diHPETE, 6-*trans*-LTB_4, and 12-*epi*-6-*trans*-LTB_4 was ≈0.8:1:1. The optimal pH for the reaction with linoleic acid, arachidonic acid, and bis-homo-γ-linolenic acid was at around 6.2, as was that with 5-HPETE. K_m values for linoleic acid, arachidonic acid, bis-homo-γ-linolenic acid, and 5-HPETE were 200, 30, 50, and 60 μM, respectively. The demonstration of the generation of the epoxide intermediate (LTA_4) in the biosynthesis of the

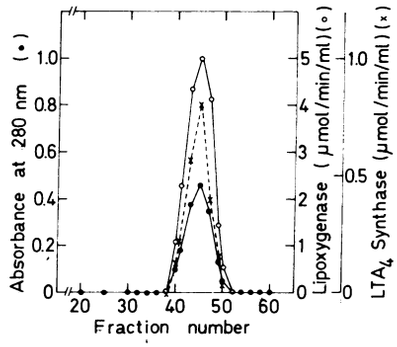

FIG. 3. Sephacryl S-300 column chromatography of the purified potato lipoxygenase. The enzyme (3.1 mg) was applied to a column of Sephacryl S-300 (1.8 × 75 cm), which was equilibrated with 50 mM potassium phosphate buffer at pH 6.8 containing 0.1 M NaCl. The enzyme was eluted with the same buffer at a flow rate of 0.2 ml/min. The eluate was collected in 1-ml fractions, and the absorbance at 280 nm (●), arachidonate 5-lipoxygenase (○), and LTA_4 synthase (×) were measured.

two isomers of 6-*trans*-LTB_4 (peaks II and III in Fig. 1) was performed as follows.

(*i*) Methanol-trapping experiments resulted in the formation of two compounds, both of which had C values of 24.2 (1% OV-101). The mass spectra of two compounds were similar, showing ions at (*m/e*): 421 (M−15), 404, 389, 325, 293, 235, 203, 171, 159 (base peak), and 129. These data were identical to those reported previously for the two 12-epimers of (5*S*)-hydroxy-12-*O*-methyl-6,8,10,14-icosatetraenoic acid (4), which are known to be formed by nonenzymatic methanolysis of LTA_4 (4).

(*ii*) Incubation of 5-HPETE with the purified enzyme under $^{18}O_2$ atmosphere demonstrated that 6-*trans*-LTB_4 and 12-*epi*-6-*trans*-LTB_4 contained ≈70% $^{16}O_2$ and ≈30% $^{18}O_2$ at the C-12 position, whereas 100% $^{18}O_2$ was incorporated into the hydroxy group at C-12 of 5,12-diHPETE (data not shown).

(*iii*) Incubation of 5-HPETE with the combination of pure lipoxygenase and highly purified LTA_4 hydrolase (LTA_4 synthase free), obtained from human leukocytes, produced LTB_4. These results, taken together, indicated that most of the two isomers of 6-*trans*-LTB_4 (>70%) were derived from LTA_4.

Further Evidence for the Involvement of Lipoxygenase in the Biosynthesis of LTA_4. The conversion of 5-HPETE to LTA_4 by lipoxygenase was further confirmed by the following experiments. The effects of various 5-lipoxygenase inhibitors on both lipoxygenation and LTA_4 synthesis were studied (Table 1). IC_{50} values for the two reactions were similar; namely, BW 755 C, phenyldisulfide, nordihydroguaiaretic acid, and 5,6-methano-LTA_4 inhibited both reactions in the range of 1 μM, whereas indomethacin had no effect. When the purified enzyme was subjected to heat treatment (40–80°C, 5 min), the activities toward three fatty acids (linoleic acid, arachidonic acid, and bishomo-γ-linolenic acid) and 5-HPETE decreased concomitantly (Fig. 4), and 50% of the remaining activities were obtained at around 55–60°C for all substrates. Furthermore, the conversion of [1-^{14}C]arachidonic acid to [1-^{14}C]5-HPETE (5-lipoxygenase) was competitively inhibited (K_i = 40 μM) by bishomo-γ-linolenic acid, as determined by TLC assay. The formation of LTA_4 as measured from the amount of two isomers of 6-*trans*-LTB_4 was inhibited by the addition of bishomo-γ-linolenic acid (50 μM, 30% inhibition; 200 μM, 62% inhibition). Considering that the lipoxygenase activity and the LTA_4 synthase activity cochromatographed on a Sephacryl S-300 column (Fig. 3), these results strongly suggest that a single enzyme isolated from potato tubers possesses both of these capacities and that LTA_4 is synthesized by this enzyme as a result of its 8-lipoxygenase activity.

Table 1. IC_{50} values of various inhibitors on lipoxygenase and LTA_4 synthase

Inhibitor	IC_{50}, μM	
	Lipoxygenase	LTA_4 synthase
BW 755 C	1	7
Nordihydroguaiaretic acid	4	13
Phenyldisulfide	5	10
5,6-Methano-LTA_4	16	5
5,8,11-Icosatriynoic acid	35	40
5,8,11,14-Icosatetraynoic acid	85	50
Indomethacin	>1,000	850
Aspirin	>1,000	>1,000

Enzyme (11.4 units/mg of protein) was preincubated with inhibitors for 5 min, and the reaction was started by the addition of substrates (linoleic acid or 5-HPETE). Lipoxygenase activity was measured by oxygen consumption.

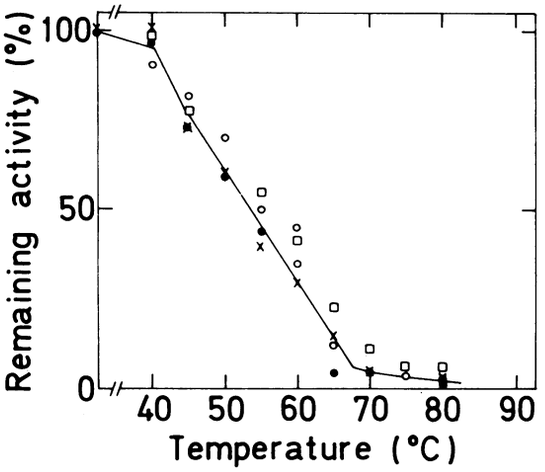

FIG. 4. Heat treatment of potato lipoxygenase. The enzyme solution (11.2 units/mg of protein) was treated for 5 min at different temperatures. After cooling the solution down in ice, the lipoxygenase activity was measured with linolenic acid (○), arachidonic acid (×), and bishomo-γ-linoleic acid (●). LTA_4 synthase was assayed (□) after the same treatment of enzyme.

DISCUSSION

Arachidonic acid was transformed by a lipoxygenase in neutrophils into 5-HPETE (2). Bishomo-γ-linolenic acid, lacking the Δ^5-double bond, is oxygenated by the same cells at C-8 (2). The transformations were also observed with a lipoxygenase preparation from potatoes. This enzyme preparation also yielded 6-*trans*-LTB_4 and 12-*epi*-6-*trans*-LTB_4 when incubated with arachidonic acid.

It was recently established that LTA_4 synthesis in neutrophils involves stereospecific elimination of the (10 *D*, pro-*R*) hydrogen at C-10 (11, 12). Because the potato lipoxygenase formed the 8-D-hydroxy derivative of bishomo-γ-linolenic acid, it followed from previous studies of the stereochemistry of hydrogen elimination in lipoxygenase catalyzed reactions (24–27) that the lipoxygenase preparation from potato should be capable of eliminating the 10 *D*-hydrogen (pro-*S*) at C-10. Because of the conjugated diene structure ($\Delta^{6,8}$) of 5-HPETE, a radical at C-6 might react with the hydroperoxy oxygen, resulting in O—O scission to yield LTA_4, instead of insertion of molecular oxygen at C-8. Therefore, it seemed conceivable that either the enzyme preparation contained two lipoxygenases involved in the formation of the 6-*trans*-LTB_4 derivatives (formed by nonenzymatic hydrolysis of LTA_4) or there was one enzyme protein with dual activities acting in concert to convert arachidonic acid by successive elimination of the *D*-hydrogens at C-7 and C-10.

To test these possibilities, the lipoxygenase from potato was purified to apparent homogeneity. The purified enzyme converted arachidonic acid to 5-HPETE and bishomo-γ-linolenic acid to 8-hydroperoxyicosatrienoic acid and small amounts of corresponding 11-hydroperoxy and 12-hydroperoxy derivatives. With 5-HPETE as substrate, 6-*trans*-LTB_4, 12-*epi*-6-*trans*-LTB_4, and 5,12-diHPETE were formed. Evidence for the intermediary formation of the epoxide LTA_4 in the biosynthesis of the two isomers of 6-*trans*-LTB_4, was obtained by trapping with methanol to yield two 12-epimers of (5*S*)-hydroxy-12-*O*-methyl-6,8,10,14-icosatetraenoic acid (cf. ref. 4). Furthermore, $^{18}O_2$ experiments demonstrated that the main part of the oxygen at C-12 of the epimeric 6-*trans*-LTB_4 derivatives originated in water, although about 30% was derived from molecular oxygen. Additional evidence for the intermediary formation of LTA_4 by the action of the purified enzyme was obtained by enzymatic transformation of the intermediate by LTA_4 hydrolase into LTB_4.

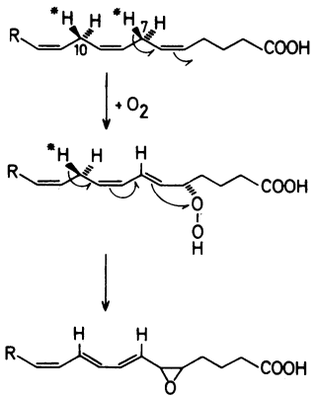

FIG. 5. Proposed reaction mechanism for the formation of LTA_4 from arachidonic acid by successive elimination of *D*-hydrogens at C-7 and C-10. R is —CH_2—CH=CH—$(CH_2)_4$—CH_3.

It was also found that 5-lipoxygenase inhibitors as BW 755 C, phenyldisulfide, nordihydroguaiaretic acid, and 5,6-methano-LTA_4 showed practically the same IC_{50} values in both oxygenation at C-5 of arachidonic acid and LTA_4 synthesis from 5-HPETE with the purified enzyme. Heat treatment resulted in a parallel decrease in the oxygenation of the polyunsaturated fatty acids and the conversion of 5-HPETE into LTA_4. Addition of bishomo-γ-linolenic acid caused inhibition of both oxygenation of arachidonic acid at C-5 (competitive inhibition) and the formation of LTA_4 from 5-HPETE.

These results strongly suggest that a single enzyme from potato tubers possesses both 5-lipoxygenase activity and LTA_4 synthase activity by virtue of its 8-lipoxygenase activity (Fig. 5). Reports on the role of a 12-lipoxygenase in the formation of 14,15-LTA_4 from 15-HPETE (28) and heme-catalyzed nonenzymatic synthesis of 14,15-LTA_4 (29) have recently appeared. Further studies are necessary to determine the nature of the enzyme(s) involved in the formation of LTA_4 in human neutrophils.

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