Properties of Highly Purified Leukotriene C_4 Synthase of Guinea Pig Lung

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Abstract

Leukotriene C_4 (LTC_4) synthase, which conjugates LTA_4 and LTA_4-methyl ester (LTA_4-me) with glutathione (GSH) to form LTC_4 and LTC_4-me, respectively, has been solubilized from the microsomes of guinea pig lung and purified 91-fold in four steps to a specific activity of 692 nmol/10 min per mg protein using LTA_4-me as substrate. LTC_4 synthase of guinea pig lung was separated from microsomal GSH S-transferase by Sepharose CL-4B chromatography and further purified by DEAE-Sephacel chromatography, agarose-butylamine chromatography, and DEAE-3SW fast-protein liquid chromatography. It was also differentiated from the microsomal GSH S-transferase, which utilized 1-chloro-2,4-dinitrobenzene as a substrate, by its heat lability and relative resistance to inhibition by S-hexyl-GSH. The K_m value of guinea pig lung LTC_4 synthase for LTA_4 was 3 μM and the V_{max} was 108 nmol/3 min per μg; the K_m values for LTA_3 and LTA_5 were similar, and the V_{max} values were about one-half those obtained with LTA_4. The conversion of LTA_4-me to LTC_4-me was competitively inhibited by LTA_3, LTA_4, and LTA_5, with respective K_i values of 1.5, 3.3, and 2.8 μM, suggesting that these substrates were recognized by a common active site. IC_{50} values for the inhibition of the conjugation of 20 μM LTA_4-me with 5 mM GSH were 2.1 μM and 0.3 μM for LTC_4 and LTC_3, respectively. In contrast, LTD_4 was substantially less inhibitory (IC_{50} > 40 μM), and LTE_4 and LTB_4 had no effect on the enzyme, indicating that the mixed type product inhibition observed was specific for sulfidopeptide leukotrienes bearing the GSH moiety.

Introduction

Leukotriene (LT)[1] C_4 is the initial sulfidopeptide leukotriene that, together with its cleavage products, LTD_4 and LTE_4, comprises the activity described as slow reacting substance of anaphylaxis (1–3). LTC_4 contracts nonvascular guinea pig smooth muscle such as ileum smooth muscle (3) and tracheal spirals (4) in vitro, and, when administered as an aerosol, compromises airflow in guinea pigs (5) and humans (6). Intravascular administration of LTC_4 to rats decreases renal blood flow by constricting the vascular smooth muscle bed and reducing blood volume by increasing venular permeability (7). Guinea pig lung (8) and human lung fragments (9) respond to immunologic activation by producing sulfidopeptide leukotrienes; these products have also been obtained from more homogeneous cell sources such as human pulmonary mast cells (10) and canine tracheal epithelial cells (11). The initial step in the biosynthesis of LTC_4 is the conversion of arachidonic acid to 5-hydroperoxy-eicosatetraenoic acid (5-HPETE) by the enzyme 5-lipoxygenase (12–14), and the same enzyme then catalyzes the conversion of 5-HPETE to LTA_4 (15, 16). LTA_4 is then conjugated with glutathione (GSH) by the enzyme LTC_4 synthase to form LTC_4 (17, 18). LTC_4 is metabolized to LTD_4 by γ-glutamyl transpeptidase (19), and to LTE_4 by a variety of dipeptidases, including one in the specific granules of human polymorphonuclear leukocytes (20).

LTC_4 synthase has been previously purified threefold from the microsomes of rat basophilic leukemia (RBL-1) cells (18). We now report the 91-fold purification of LTC_4 synthase from guinea pig lung microsomes and its kinetic characterization and substrate specificity.

Methods

Materials. LTA_4 methyl ester (LTA_4-me) (21) and 14,15-LTA_4-me (22) were provided by Dr. E. J. Corey of Harvard University (Cambridge, MA) and Dr. J. Rokach of Merck-Frosst, Canada Inc., respectively. The methyl esters of LTA_3 (23), LTA_5 (24), 5-epi-LTA_4, 6-epi-LTA_4, and 5-epi-, 6-epi-LTA_4 (25) were synthesized as described, and the lithium salts of these epoxide-leukotrienes were prepared (17). [14,15-^{3}H]LTA_4-me (50 Ci/mmol) was obtained from New England Nuclear (Boston, MA); LTC_4, LTD_4, LTE_4, and LTC_4-me were synthesized as previously described (26). Arachidonic acid (NuCheck Prep, Inc., Elysian, MN), prostaglandin B_2 (PGB_2), 1-chloro-2,4-dinitrobenzene, GSH, 4-(2-hydroxyethyl)-1-piperazine ethanesulfonic acid (Hepes), bovine serum albumin, Triton X-102, deoxycholic acid (Sigma Chemical Co., St. Louis, MO), glycerol (Aldrich Chemical Co., Milwaukee, WI), 2-mercaptoethanol (Eastman Kodak Co., Rochester, NY), Sepharose CL-4B, diethylaminoethyl (DEAE)-Sephacel, Superose 12 (Pharmacia Fine Chemicals, Inc., Piscataway, NJ), DEAE-3SW (2.15 × 15 cm) (Bio-Rad Laboratories, Richmond, CA), and Agarose-butylamine (P.-L. Biochemicals, Milwaukee, WI) were purchased as noted.

Preparation of microsomes from guinea pig lung. Lungs (~ 100 g from 30 animals) were collected in 50 ml of ice-cold 20 mM Tris-HCl buffer, pH 8.0, containing 1 mM EDTA and 5 mM 2-mercaptoethanol. The lungs were chopped into small pieces with scissors and the fragments rinsed once in ~ 250 ml of homogenization buffer and then resuspended in 5-vol wet weight of buffer and disrupted using a Tissuemizer (model SDT-1810; Tekmar Co., Cincinnati, OH). All subsequent steps were carried out at 4°C. The homogenate was centrifuged

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1. *Abbreviations used in this paper:* Buffer A, 20 mM Tris-HCl, pH 8.0, 1 mM EDTA, 5 mM 2-mercaptoethanol, 10% glycerol; FPLC, fast protein liquid chromatography; 5-HPETE, 5-hydroperoxy-eicosatetraenoic acid; LT, leukotriene; LTA_4, LTC_4, LTD_4, LTE_4, leukotrienes A_4, C_4, D_4, and E_4, respectively; LTA_4-me, LTA_4 methyl ester; RBL-1 rat basophilic leukemia.

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the supernatant was filtered through cheesecloth. The filtrate was centrifuged at 10,000 *g* for 10 min, and the resulting supernatant was centrifuged at 105,000 *g* for 60 min. The supernatant (cytosol) was removed, and the pellet was washed by homogenization, using a Potter-Elvejhem homogenizer in the same buffer, centrifuged at 105,000 *g* for 1 h, and resuspended in 1 vol of the same buffer by homogenization (microsomes). Protein concentration was determined by the method of Lowry et al. (27) after precipitation of the protein with 6% trichloroacetic acid and 0.012% deoxycholic acid. Bovine serum albumin was used as a standard.

Enzyme assays. For assay of LTC_4 synthase, the reaction contained 50 mM Hepes buffer at pH 7.6, 20 μM LTA_4-me (in 5 μl ethanol), 5 mM GSH and enzyme in a final volume of 0.5 ml. Assays were incubated for 10 min at 24°C except for the determination of kinetic parameters, for which the incubation time was shortened to 3 min to reflect the initial velocity. The reaction was terminated by the addition of 0.5 ml of a solvent mixture of methanol/acetonitrile/acetic acid (50:50:1, vol/vol/vol) containing PGB_2 (100 ng) as an internal standard (18). The resulting mixture was centrifuged at 18,000 *g* for 10 min. The extract (supernatant) of the reaction mixture was then injected onto an Ultrasphere-ODS column (5-μm particles, 4.6 × 250 mm) which was eluted with a solvent mixture of methanol/acetonitrile/water/acetic acid, pH 5.6 (13:5:8.1:0.9) (vol/vol/vol/vol); the column was run at a flow rate of 1 ml/min, and absorbance at 280 nm was continuously monitored. Retention times of PGB_2 and LTC_4-me in this system were 8.8 min and 12.2 min, respectively. One unit of LTC_4 synthase activity was defined as forming 1 nmol of LTC_4-me per 10 min at 24°C. When LTA_4 (lithium salt in 5 μl ethanol) was used as a substrate, a solvent mixture of methanol/acetonitrile/water/acetic acid, pH 5.6 (5:5:8.1:0.9) (vol/vol/vol/vol) was used. Retention times of the reference standards were 15.4 min (LTC_3), 9.1 min (LTC_4), 6.9 min (LTC_5), 8.3 min (14, 15-LTC_4), 9.3 min (5-epi-LTC_4), 9.4 min (6-epi-LTC_4), 9.2 min (5-epi-, 6-epi-LTC_4) and 16.4 min (PGB_2, internal standard). GSH-S-transferase activity was measured spectrophotometrically according to the method of Habig et al. (28) at pH 6.5 with 1-chloro-2,4-dinitrobenzene (1 mM) and GSH (1 mM) as substrates.

Results

Subcellular localization of LTC_4 synthase. The subcellular localization of LTC_4 synthase in guinea pig lung tissue homogenate was determined after differential centrifugation. Each subcellular fraction was incubated with 20 μM LTA_4 in the presence of 5 mM GSH for 10 min at 24°C, and the extract of the reaction mixture was analyzed by reverse-phase high performance liquid chromatography (RP-HPLC). The 10,000 *g* pellet (Fig. 1 *A*) contained a small amount of the total activity of the homogenate. About 75% of the LTC_4 synthase activity was recovered in the microsomal fraction (Fig. 1 *B*), representing an 11-fold increase of specific activity over the homogenate. LTC_4 was not produced if either GSH or the microsomes were omitted from the reaction mixture. Barely detectable amounts of LTC_4 were formed in the cytosol (Fig. 1 *C*). When the GSH S-transferase activity of the subcellular fractions was determined, with 1 mM 1-chloro-2,4-dinitrobenzene and 1 mM GSH as substrates, 91% of the total activity of the homogenate was recovered in the cytosolic fraction. The 10,000 *g* pellet and the microsomes accounted for 2% and 5% of the GSH S-transferase activity, respectively.

Purification of LTC_4 synthase. All isolation procedures were conducted at 4°C, and LTA_4-me was used as the substrate.

Glycerol was added to microsomal suspensions of about 250 mg of protein in 70 ml at a final concentration of 20% (wt/vol) and Triton X-102 and deoxycholic acid were each added to a final concentration of 0.4%. The mixture was

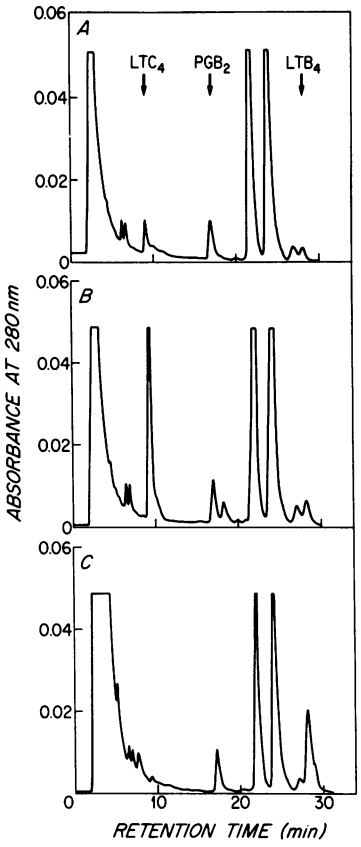

Figure 1. Biosynthesis of LTC_4 by incubation of subcellular fractions of guinea pig lung with 20 μM LTA_4 and 5 mM GSH. (*A*) 10,000 *g* pellet (130 μg of protein); (*B*) 105,000 *g* pellet (190 μg of protein); (*C*) 105,000 *g* supernatant (704 μg of protein). Products were resolved by RP-HPLC and quantitated by integrated optical density at 280 nm. Retention times of authentic compounds are indicated by arrows.

stirred for 30 min followed by centrifugation at 105,000 *g* for 1 h. LTC_4 synthase was solubilized with a recovery of 51±7.2% (mean±SD, n = 4) of the initial activity of the microsomes. Although the enzyme could be solubilized with 0.4% Triton X-102 alone, the combination of a nonionic and an ionic detergent was used routinely for solubilization; this prevented the formation of mixed aggregates that interfered with the gel filtration chromatography.

35 ml of the supernatant, containing solubilized enzyme, was applied onto a Sepharose CL-4B column (4.4 × 50 cm) that had been equilibrated with 20 mM Tris-HCl buffer at pH 8.0, 1 mM EDTA, 5 mM 2-mercaptoethanol, and 10% glycerol (buffer A) containing 0.1% Triton X-102 and 0.1% deoxycholic acid. The column was eluted at a flow rate of 45 ml/h. Microsomal LTC_4 synthase from the solubilized guinea pig lung was resolved (V_e = 550 ml) from GSH S-transferase (V_e = 630 ml) by its greater apparent size (Fig. 2).

The peak fractions containing LTC_4 synthase activity (Fig. 2) were pooled and applied to a DEAE-Sephacel column (2.5 × 4 cm) that had been equilibrated with buffer A containing 0.1% Triton X-102 and 0.1% deoxycholic acid. The column was washed with two column volumes of the same buffer and the flow-through fractions were collected (90 ml). LTC_4 synthase appeared in the effluent, whereas > 90% of total protein was retained, resulting in a 30-fold purification achieved in this step.

The DEAE-Sephacel flow-through was applied to an agarose-butylamine column (2.5 × 10-cm) that had been equilibrated with buffer A. The column was washed with two column volumes of the same buffer and then eluted with buffer A containing 0.1% Triton X-102 and 0.1% deoxycholic acid. 6-ml fractions were collected and the eluate (30 ml) was frozen at −70°C. A duplicate purification was then performed and

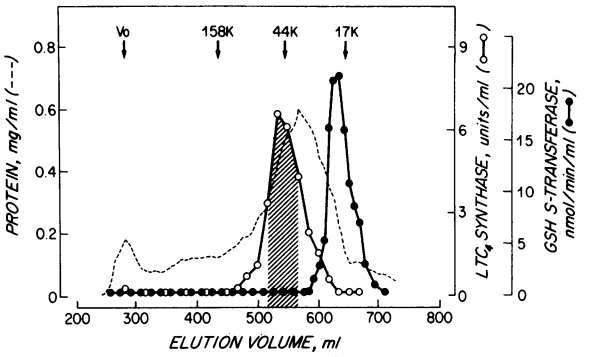

Figure 2. Gel filtration of 66 mg of the solubilized microsomes of guinea pig lung on a Sepharose CL-4B column (4.4 × 50 cm) in buffer A containing 0.1% deoxycholic acid and 0.1% Triton X-102. 8.5-ml fractions were collected and protein concentration (- - -), GSH S-transferase activity (●), and LTC_4 synthase activity (o) were determined. The striped bar indicates the fractions pooled for further purification.

the two preparations of the enzyme were pooled to a 60-ml total volume.

The pooled enzyme preparation was injected onto a DEAE-3SW fast protein liquid chromatography (FPLC) column (2.15 × 15 cm) that was previously equilibrated with 50 mM potassium phosphate buffer at pH 8.0 containing 1 mM EDTA, 5 mM 2-mercaptoethanol, 10% glycerol, and 0.1% Triton X-102. The column was washed at a flow rate of 3 ml/min and was developed in a linear gradient (120 ml) of 0 to 0.2 M NaCl and 0 to 0.1% deoxycholic acid in the same buffer. LTC_4 synthase eluted at a salt concentration of 0.16 M and a deoxycholate concentration of 0.08% (Fig. 3). Gradients of both salt and deoxycholate were required to prevent the elution of LTC_4 synthase Triton micelles along with the bulk of the protein. Peak active fractions were pooled (15 ml), frozen at −70°C, and used as the source of purified LTC_4 synthase.

The yields from a representative purification of LTC_4 synthase from the microsomes of guinea pig lung are tabulated in Table I. In four separate purifications the final average specific activity was 697 U/mg of protein (533-844 U/mg) with an overall yield of 3% (2–6%, $n = 4$).

Properties of LTC_4 synthase. When 6 ml of the highly purified enzyme was applied onto a 2.3 × 46-cm Superose 12 column, and eluted at a flow rate of 1 ml/min, a single peak of LTC_4 synthase activity filtered in a M_r range of 54,000-58,000 (Fig. 4 *A* and *B*). There was no appreciable loss of purified LTC_4 synthase activity for 2 wk upon storage at −70°C,

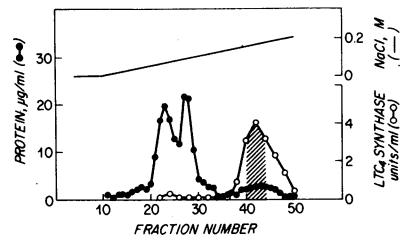

Figure 3. Elution profile of LTC_4 synthase from a DEAE-3SW column (2.15 × 15 cm) loaded with pooled active fractions from the agarose-butylamine step. 3-ml fractions were collected and protein concentrations (●) and LTC_4 synthase activity (o) were determined. The bar indicates the fractions used as the purified enzyme.

Table I. Representative Purification of Guinea Pig LTC_4 Synthase

Step	Protein	Total activity	Yield	Specific activity	Purification
	mg	*U*	*%*	*U/mg*	*fold*
Microsomes	251	1,907	100	7.6	1
Solubilized	184	1,140	60	6.2	0.8
Sepharose 4B	74	398	21	5.4	0.7
DEAE-Sephacel	6.0	986	51	164	22
Agarose-butylamine	2.8	640	34	229	30
DEAE (FPLC)	0.078	54	3	692	91

whereas approximately half of the enzyme activity was lost in 2 d at 4°C.

The time course of the LTC_4 synthase reaction using 1.8 μg of purified enzyme was linear for the first 5 min, after which the reaction rate gradually decreased over the next 25 min (Fig. 5 *A*). However, further addition of 20 μM LTA_4-me at 20 min increased the rate of product formation appreciably, indicating that the LTC_4 synthase was not substantially inactivated during catalysis. The addition of bovine serum albumin (1 mg/ml) to the reaction mixture did not alter the time course of the LTC_4 synthase reaction. The rate of product formation was proportional to the amount of enzyme protein added to the reaction mixture from ~ 1.5 to 4.0 μg protein (Fig. 5 *B*), and all reactions were carried out within this range. The reaction rate increased as the pH of the reaction mixture was raised (Fig. 6), but above pH 8 nonenzymatic conjugation of GSH with LTA_4-me occurred. Thus, pH 7.6 was used routinely for the assays because at this pH nonenzymatic conjugation was negligible (18). S-hexyl-GSH inhibited microsomal GSH S-transferase obtained at the Sepharose CL-4B chromatography step in a dose-dependent manner over the range of 0 to 50 μM with an IC_{50} of 4.6 μM, whereas the purified LTC_4 synthase was unaffected at this concentration and was inhibited only 17% at 50 μM. When both enzyme preparations were kept for 5 min over a range of temperatures from 25 to 60°C, LTC_4 synthase activity decreased by 44% at 35°C and by 96% at 45°C, whereas microsomal GSH S-transferase was stable up to 40°C and lost only 20% of the activity at 45°C.

The effect of GSH concentration on the LTC_4 synthase

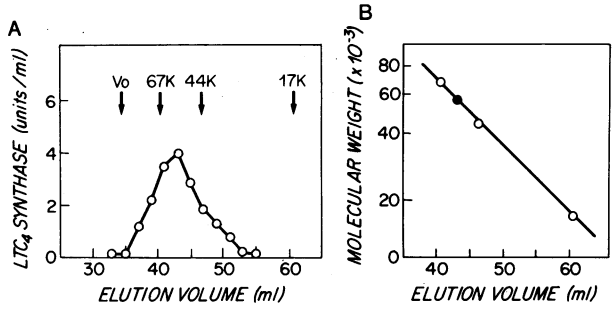

Figure 4. (*A*) Superose 12 gel filtration of LTC_4 synthase in buffer A containing 0.1% deoxycholate and 0.1% Triton X-102. 2-ml fractions were collected. (*B*) Determination of M_r of LTC_4 synthase. M_r standards (o) were: bovine serum albumin (67,000 M_r), ovalbumin (45,000 M_r) and myoglobin (17,000). LTC_4 synthase is represented by closed circles (●).

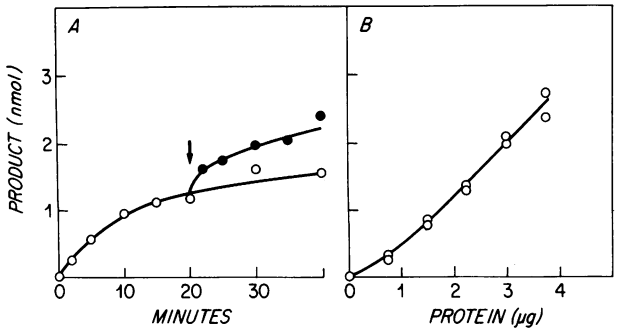

Figure 5. Time course (*A*) and protein dependency (*B*) of LTC_4 synthase reaction. (*A*) Purified enzyme was incubated with 20 μM LTA_4-me for the periods indicated (○), and at 20 min (*arrow*) another 20 μM of LTA_4-me was added (●); (*B*) Varying amounts of the purified enzyme were incubated for 10 min with 20 μM LTA_4-me and 5 mM GSH.

reaction with 20 μM LTA_4-me and purified enzyme revealed saturation above 10 mM GSH and a K_m value of 2.3 mM by Lineweaver-Burk plots (Fig. 7).

Substrate specificity and kinetics of LTC_4 synthase. Fig. 8 shows the specificity of a variety of epoxide substrates for LTC_4 synthase, when their concentration was varied in the presence of 5 mM GSH. The apparent K_m and V_{max} values for the highly purified enzyme were calculated to be 3 μM and 108 nmol/3 min mg, respectively, by Lineweaver-Burk plots. LTA_3 and LTA_5 were about 50% as active as LTA_4 and had V_{max} values of 48 and 54 nmol/3 min/mg, and K_m values of 4.3 μM and 2.6 μM, respectively. The methyl ester of LTA_4 was found to have a K_m value of 16 μM and V_{max} of 420 nmol/3 min/mg. 14,15-LTA_4 was converted to its GSH conjugate by the LTC_4 synthase with K_m and V_{max} values of 77 μM and 204 nmol/3 min per mg, respectively. LTC_4 synthase also catalyzed the conjugation of GSH with the epimers of LTA_4 to produce corresponding epimers of LTC_4, and the K_m and V_{max} values for these epimers were 38 μM for 5-epi-LTA_4 (V_{max}, 93 nmol/3 min per mg), 24 μM for 6-epi-LTA_4 (V_{max}, 162 nmol/3 min per mg) and 27 μM for 5-epi, 6-epi-LTA_4 (V_{max}, 102 nmol/3 min per mg).

Inhibition of LTC_4 synthase. The action of purified LTC_4 synthase upon 20 μM LTA_4-me in the presence of 5 mM GSH was inhibited in a dose-dependent manner by LTA_3, LTA_4, and LTA_5 (Fig. 9 *A*). The inhibition was determined kinetically in the presence of 0.5 μM LTA_3, 3 μM LTA_4, and 3 μM LTA_5 and analyzed by double-reciprocal plots (Fig. 9 *B*). The inhibition was found to be competitive and the K_i values were

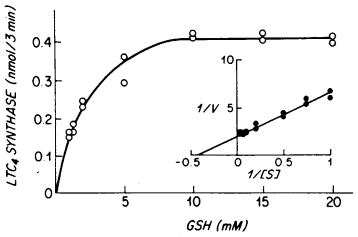

Figure 7. Effect of GSH concentration on LTC_4 synthase activity (○) in the reaction mixture with 20 μM LTA_4-me. The inset represents Lineweaver-Burk plots of corresponding points (●).

calculated to be 1.5 μM for LTA_3, 3.3 μM for LTA_4, and 2.8 μM for LTA_5, respectively.

Inhibition of the LTC_4 synthase reaction by LTC_4, the product of the enzyme reaction with LTA_4, was dose dependent and the IC_{50} value was estimated to be 2.1 μM (Fig. 10). Preincubation of the enzyme with 2 μM LTC_4 for up to 30 min did not alter the degree of inhibition, suggesting a reversible inhibition. 14,15-LTC_4 also inhibited LTC_4 synthase reaction with an IC_{50} value of ~ 6 μM. LTC_5, LTD_4, and LTC_4-me showed a less inhibitory effect on the enzyme, inhibiting the reaction between 30 and 40% at a concentration of 10 μM. Arachidonic acid and 5-hydroxy-6,8,11,14-eicosatetraenoic acid were less inhibitory. LTE_4 and LTB_4 did not inhibit the reaction at 10-μM concentrations. In a separate experiment, in which the reaction of LTC_4 synthase with 20 μM LTA_4-me was carried out with varying concentrations of LTC_4 from 4-40 μM and analyzed by Lineweaver-Burk plots, the inhibition was of the mixed type with a K_i of 2.7 μM.

Discussion

LTC_4 synthase of guinea pig lung microsomes was solubilized and purified to a specific activity of 697 U/mg by a four-step procedure that included Sepharose 4B filtration, DEAE-Sephacel chromatography, agarose-butylamine chromatography, and DEAE-3SW FPLC (Figs. 2 and 3; Table I). The utilization of a second detergent, deoxycholate, was critical in preventing the formation of large aggregates that resulted in the elution of enzyme activity in the void volume of the Sepharose CL-4B column. The final enzyme preparation was unstable after solubilization, especially at acidic pH. Therefore chromatography was conducted in the pH range of 7.6 to 8.0 and storage was at −70°C. The addition of glycerol and sulfhydryl compounds such as 2-mercaptoethanol partially protected the enzyme from inactivation, resulting in a half-life of 2 d for the final preparation of purified enzyme at 4°C and complete stability at −70°C for at least 2 wk. Gel filtration of purified LTC_4 synthase activity by Superose 12 gel filtration chromatography

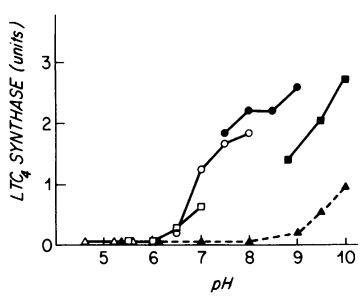

Figure 6. pH profile of LTC_4 synthase reaction. Purified enzyme was incubated with 20 μM LTA_4-me and 5 mM GSH at various pHs, which were achieved with sodium acetate (△), potassium phosphate (□), Hepes (○), Tris (●), and glycine-NaOH (■) buffers at a concentration of 50 mM. Product formation in the absence of enzyme protein is depicted by the broken line (▲).

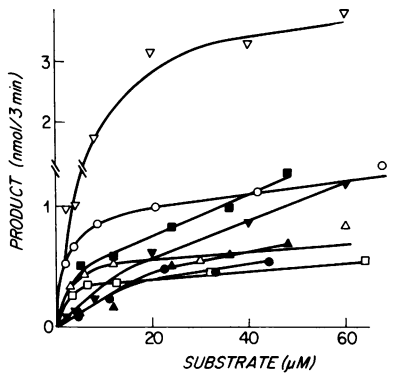

Figure 8. Substrate specificity of the LTC_4 synthase of guinea pig lung. Various epoxides were incubated with the purified enzyme for 3 min. The substrates employed were LTA_4 (▼), LTA_4-me (▽), LTA_3 (□), LTA_5 (△), 14,15-LTA_4 (○), 5-epi-LTA_4 (●), 6-epi-LTA_4 (■), and 5-epi-, 6-epi-LTA_4 (▲).

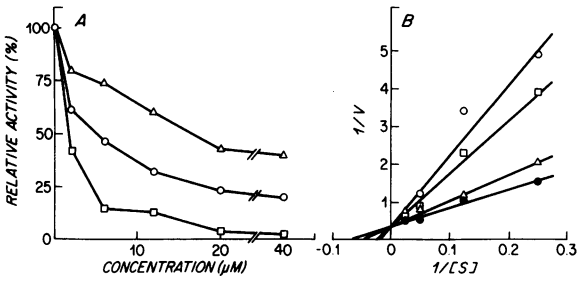

Figure 9. (*A*) Inhibition of LTC_4 synthase activity during a 3-min incubation with LTA_4-me and GSH by LTA_3 (□), LTA_4 (○), and LTA_5 (△). The percent relative activity is expressed as the percentage of the LTC_4 synthase activity without any added compound which was 1.5 nmol/3 min; (*B*) Double-reciprocal plots of the data in which the incubations were carried out in the absence (●) or presence of 0.5 μM LTA_3 (□), 3 μM LTA_4 (○), and 3 μM LTA_5 (△).

indicated an apparent M_r of 54,000–58,000 (Fig. 4). The specific activity of the guinea pig lung microsomal LTC_4 synthase of 697 U/mg appears to be comparable to that of the two most active purified rat liver cytosolic isoenzymes of GSH S-transferase for conversion of LTA_4-me to LTC_4-me in the presence of GSH (29).

Subcellular localization of LTC_4 synthase and GSH S-transferase as examined using LTA_4 and 1-chloro-2,4-dinitrobenzene as substrates, respectively, revealed that LTC_4 synthase was associated with the microsomal fraction (Fig. 1), whereas GSH S-transferase was mostly present in the cytosolic fraction in guinea pig lung. Similar results were previously reported for RBL-1 cells (17, 18) and guinea pig lung (30) and indicate that tissue sources can be used for enzyme purification, thereby somewhat alleviating the problems of insufficient material for high purification and detailed kinetic studies. It has been reported that the microsomal GSH S-transferase of rat liver with the minimum M_r of 17,237 (31) presents as a trimer in the enzyme-detergent complex (129,300 M_r). The microsomal GSH S-transferase of guinea pig lung had an estimated M_r of 25,000–30,000 by gel filtration and was separated from microsomal LTC_4 synthase by gel filtration (Fig. 2). Whether the microsomal GSH transferase of guinea pig lung and LTC_4 synthase have similar molecular weights, but interact with detergents to form different-sized oligomers which are resolved by gel filtration is not known. The distinct natures of these microsomal enzymes were also demonstrated by their differential sensitivity to S-hexyl-GSH, an inhibitor of GSH S-transferase (32), and to modest heat.

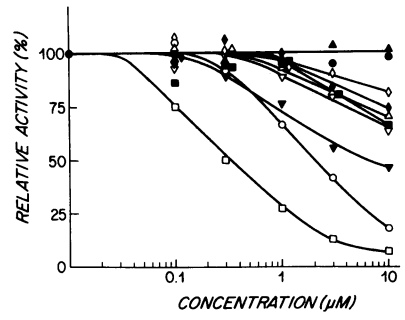

Figure 10. Inhibition of LTC_4 synthase activity with LTA_4-me and GSH by LTC_3 (□), LTC_4 (○), LTC_5 (△), LTC_4-me (▽), 14,15-LTC_4 (▼), LTD_4 (■), LTE_4 (●), LTB_4 (▲), 5-hydroxy-6,8,11,14-eicosatetraenoic acid (◇) and arachidonic acid (◆). In the experiments with LTC_4-me, LTE_4, LTB_4 the reaction mixture was supplemented with [14,15-^{3}H]LTA_4-me and the product was quantitated by liquid scintillation counting. The percent relative activity is expressed as the percentage of LTC_4 synthase activity without any added compound.

The time-course of LTA_4 conjugation with GSH showed a hyperbolic curve (Fig. 5 *A*), indicating that the reaction rate gradually decreased after 5 min. The reaction of the LTC_4 synthase with LTA_4 and GSH is apparently longer than that of LTA_4 hydrolase, which ceases to yield LTB_4 within 1–2 min (33, 34) because of irreversible inactivation. It should be noted that LTC_4 synthase, unlike LTA_4 hydrolase (33, 34), remained active during the course of reaction, because further addition of the substrate (LTA_4-me) augmented the production of LTC_4-me (Fig. 5 *A, arrow*). Since the half-life of LTA_4 was estimated to be < 0.1 min at 25°C (pH 7.4) (35), this unstable epoxide might be stabilized in its reaction with microsomal LTC_4 synthase by either the enzyme protein or the detergents that were present in the enzyme preparation. LTC_4 synthase exhibited a broad alkaline pH optimum (Fig. 6), and the greater activity of LTC_4 synthase at alkaline pH might be influenced by the improved stability of the substrate, as well as the enzyme, in this pH range relative to acidic conditions (36). The apparent K_m value (2.3 mM) of guinea pig LTC_4 synthase for GSH (Fig. 7) is comparable to that (3-6 mM) of LTC_4 synthase of RBL-1 cells (18).

Among the compounds examined as substrates for LTC_4 synthase (Fig. 8), LTA_4-me showed the highest V_{max}, and this may be explained by the hydrophobic nature of the ester as compared with the salt, since the protein is a membrane-associated enzyme and presumably has a hydrophobic region. However, the natural substrate, LTA_4, showed a lower K_m value than that of LTA_4-me. The fact that the enzyme reacts with LTA_3 and LTA_5 gives an enzymological basis for the reports demonstrating the formation of LTC_3 (37, 38) and LTC_5 (39) from mouse mastocytoma and RBL-1 cells. In view of the fact that 5-epi-LTA_4 is less active than 6-epi-LTA_4, the configuration of C-5 of LTA_4 may be more important than C-6 for the recognition unit of LTC_4 synthase. The competitive inhibition by LTA_3, LTA_4, and LTA_5 of LTC_4 synthase reaction with LTA_4-me (Fig. 9) suggests that the conjugation of GSH with these substrates is catalyzed at the same active site of the enzyme. LTA_3 (40) and LTA_5 (41) were also shown to inhibit LTA_4 hydrolase of human neutrophils. The rank order of potency of the LTA_4 hydrolase inhibition is the same as that of LTC_4 synthase, namely, LTA_3 is the most potent inhibitor.

LTC_3 and LTC_4 were potent inhibitors of LTC_4 synthase using LTA_4-me as a substrate (Fig. 10). Since LTD_4, LTE_4, and LTB_4 showed significantly less or negligible inhibitory effects on LTC_4 synthase, the inhibition by LTC_4 appears to be product inhibition of the enzyme and is a property shared with most xenobiotic conjugating GSH transferases (42).

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