Identification and Characterization of a Novel Human Microsomal Glutathione S-Transferase with Leukotriene C₄ Synthase Activity and Significant Sequence Identity to 5-Lipoxygenase-activating Protein and Leukotriene C₄ Synthase*

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5-Lipoxygenase-activating protein (FLAP) and leukotriene C₄ (LTC₄) synthase, two proteins involved in leukotriene biosynthesis, have been demonstrated to be 31% identical at the amino acid level. We have recently identified and characterized a novel member of the FLAP/LTC₄ synthase gene family termed microsomal glutathione S-transferase II (microsomal GST-II). The open reading frame encodes a 16.6-kDa protein with a calculated pl of 10.4. Microsomal GST-II has 33% amino acid identity to FLAP, 44% amino acid identity to LTC₄ synthase, and 11% amino acid identity to the previously characterized human microsomal GST (microsomal GST-I). Microsomal GST-II also has a similar hydrophobicity pattern to FLAP, LTC₄ synthase, and microsomal GST-I. Fluorescent in situ hybridization mapped microsomal GST-II to chromosomal localization 4q28–31. Microsomal GST-II has a wide tissue distribution (at the mRNA level) and was specifically expressed in human liver, spleen, skeletal muscle, heart, adrenals, pancreas, prostate, testis, fetal liver, and fetal spleen. In contrast, microsomal GST-II mRNA expression was very low (when present) in lung, brain, placenta, and bone marrow. This differs from FLAP mRNA, which was detected in lung, various organs of the immune system, and peripheral blood leukocytes, and LTC₄ synthase mRNA, which could not be detected in any tissues by Northern blot analysis. Microsomal GST-II and LTC₄ synthase were expressed in a baculovirus insect cell system, and microsomes from SF9 cells containing microsomal GST-II or LTC₄ synthase were both found to catalyze the production of LTC₄ from LTA₄ and reduced glutathione. Microsomal GST-II also catalyzed the formation of another product, displaying a conjugated triene UV absorption spectra with a maximum at 283 nm, suggesting less catalytic stereospecificity compared with LTC₄ synthase. Also, the apparent Kₘ for LTA₄ was higher for microsomal GST-II (41 μM) than LTC₄ synthase (7 μM). In addition, unlike LTC₄ synthase, microsomal GST-II was able to catalyze the conjugation of 1-chloro-2,4-dinitrobenzene with reduced glutathione. Therefore, it is proposed that this novel membrane protein is a member of the microsomal glutathione S-transferase family, also including LTC₄ synthase, with significant sequence identities to both LTC₄ synthase and FLAP.

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5-Lipoxygenase-activating protein (FLAP) and leukotriene (LT) C₄ synthase are both proteins involved in the biosynthesis of leukotrienes. Leukotrienes are biologically active compounds that function as mediators of various inflammatory processes, such as leukocyte chemotaxis, increased vascular permeability, smooth muscle constriction, and increased mucus secretion (1, 2). Cellular leukotriene biosynthesis is initiated by a rise in intracellular calcium, which induces the release of arachidonic acid from different phospholipids by various classes of phospholipases (3–6). Calcium also initiates the translocation of arachidonate 5-lipoxygenase to the nuclear membrane (7, 8). Subsequently, 5-lipoxygenase catalyzes a two-step reaction from arachidonic acid to the unstable epoxide LTA₄ via 5-hydroperoxyeicosatetraenoic acid (9). This reaction in intact cells requires the presence of FLAP (10) an 18-kDa protein localized to the nuclear membrane (8, 11). FLAP has been demonstrated to bind arachidonic acid and to increase the efficiency of 5-lipoxygenase conversion of 5-hydroperoxyeicosatetraenoic acid to LTA₄ (12, 13); however, no enzymatic function has been described for FLAP. The epoxide LTA₄ can be hydrolyzed to LTB₄ by the cytosolic protein LTA₄ hydrolase or conjugated with reduced glutathione by LTC₄ synthase to LTC₄ (1). LTC₄ synthase is a microsomal 16.6-kDa polypeptide that is enzymatically active as a homodimer (14) and was recently cloned independently by two groups (15, 16).

5-Lipoxygenase and FLAP expression is restricted to various myeloid cells, B lymphocytes, and pancreatic acinar cells (1, 17, 18). LTC₄ synthase activity has been described in eosinophils, basophils, mast cells, and certain phagocytic mononuclear cells (19–22). Also, human endothelial cells, vascular smooth muscle cells, and platelets all express LTC₄ synthase activity without concomitant expression of 5-lipoxygenase. The formation of LTC₄ in these cells is therefore dependent on the transcellular metabolism of LTA₄ e.g. by interaction with activated neutrophils (23–28). LTC₄ synthase activity has also been described in certain leukemic cell lines such as KG-1 cells, THP-1 cells, U-937 cells, and HL-60 cells (14, 29–31). Furthermore, an increased LTC₄ formation has been reported in leukocytes from patients with chronic myelogenous leukemia (32) and in experimental glomerulonephritis (33). Although various cytotoxic...
glutathione S-transferases (GSTs) may conjugate LTA₄ with glutathione to form LTC₄. LTC₄ synthase has been defined as a microsomal protein distinct from human cytosolic and microsomal GSTs (37–39). Successful attempts to purify LTC₄ synthase in the KCl-1 myeloid cell line as well as dimethyl sulfoxide-differentiated U937 cells have confirmed LTC₄ synthase as being a distinct membrane protein with no activity toward 1-chloro-2,4-dinitrobenzene (substrate for the α, μ, π, and microsomal classes of GSTs) or p-nitrobenzylchloride (substrate for the θ class of GSTs) as well as lack of recognition by specific antisera raised against α, μ, π, and microsomal GSTs (29, 30). Cytosolic GSTs are active as homo- or heterodimers of subunits of ~25 kDa, whereas microsomal GST is active as a trimer with a subunit size of 17 kDa (40, 41). Both cytosolic and microsomal GSTs are heavily expressed in the liver but are also found in various tissues such as kidney, lung, skeletal muscle, intestine, adrenals, heart, pancreas, and testes (40, 41). The 1-chloro-2,4-dinitrobenzene conjugation activity of both rat and human microsomal GSTs is activated by N-ethylmaleimide (42–44). The cDNAs of both the rat and the human microsomal GSTs contain an open reading frame encoding a 154-amino acid polypeptide, and the two proteins show 85% amino acid identity (45). These enzymes will be referred to as human or rat microsomal glutathione S-transferase I (microsomal GST-I). The microsomal GST-I from both species has a wide specificity for lipophilic and electrophilic substrates; however, LTA₄ is a poor substrate for microsomal GST-I (39, 44, 46, 47), which therefore should not contribute to the LTC₄ synthase activity reported in various tissues and cells. The biological functions of GSTs are attributed to the detoxification of xenobiotics and metabolism of drugs as well as protection from oxidative stress caused by lipid peroxidation (40, 41, 48). In this report we describe a novel protein with characteristics in common with FLAP, LTC₄ synthase, and microsomal GST-I.

MATERIALS AND METHODS

Cells—Spodoptera frugiperda Sf9 cells were obtained from Invitrogen and cultured in Grace’s insect media supplemented with fetal bovine serum (10%), gentamicin (50 μg/ml), and fungizone (2.5 μg/ml). The cells were cultured at 28° C, and the stock cell concentration was maintained between 0.5 and 3 × 10⁶ cells/ml. Isolation of granulocytes and mononuclear cells from human blood was performed essentially as described previously (49). Briefly, 50 ml of whole blood, obtained from a healthy donor, was mixed with dextran (0.5% w/v, final concentration). After sedimentation of the erythrocytes for 30 min, the resulting plasma was centrifuged at 200 × g for 15 min. The cell pellet was resuspended in phosphate-buffered saline, pH 7.4 (Dulbecco’s formula) and washed twice at 200 × g for 10 min. Erythrocytes were removed by hypotonic lysis with distilled water. Subsequently, the leukocytes were applied on a discontinuous density gradient (Ficoll–Isopaque) and centrifuged at 600 × g for 30 min (49). Mononuclear cells and granulocytes were collected and washed once. Viability was better than 98% as determined by trypan blue exclusion. The cell concentration was adjusted to 1.5 × 10⁶/ml.

Sequencing, Cloning, and Construction of Recombinant Baculovirus—A TBLASTN search of the GenBank™ data base using the FLAP peptide sequence revealed similarity with the sequence deposited by the WashU-Merck EST project with an accession number of H59143. The sequence of the novel cDNA (termed microsomal GST-II) was confirmed on both strands according to the Sanger dideoxy chain termination method (50), using the PRISMTM ready reaction dye-deoxy terminator cycle sequencing kit and an ABI model 373 DNA sequencer. Oligonucleotides for sequencing and RT-PCR were obtained from Research Genetics (Huntsville, AL). The insert cDNA sequence was released from the pT7T3D vector by an EcoRI/HindIII double digest, end-filled with Klenow, and blunt-end-ligated into the Stul–cut multiple cloning site of the pFastBac vector (Life Technologies, Inc.). Virus was constructed according to the Bac-to-Bac Baculovirus expression systems, as described by the manufacturer’s instructions (Life Technologies, Inc.). Also, a cDNA for LTC₄ synthase was kindly provided by K. Scoogan.

Chromosomal Localization of Microsomal GST-II by Fluorescence in Situ Hybridization—Hybridization screening of a P1 artificial chromosome (PAC) library followed by fluorescence in situ hybridization was performed by Bios Laboratories, Inc. The initial PAC library screening was facilitated using a human microsomal GST-II cDNA obtained by random priming. Four positive PAC clones were obtained and confirmed by Southern blotting of the HaelII-digested DNA. The clone with the best yield (PAC clone 180H14) was used in the subsequent chromosomal localization. The PAC clone 180H14 was labeled with digoxigenin dUTP by nick translation. The labeled probe was combined with human DNA and salmon sperm DNA and hybridized to pronase-treated chromosomes obtained from PHA-stimulated peripheral blood lymphocytes in a solution containing 50% formamide, 10% dextran sulfate, and 2 × SSC. A biotin-labeled probe specific for the centromere of chromosome 4 was cohybridized with clone 180H14. Hybridization signals were detected with antidigoxigenin antibodies conjugated with rhodamine or fluoresceinated avidin, followed by counterstaining with 4′,6-diamidino-2-phenylindole. Northern Blot Analysis—Northern blot of human multiple tissue blots (Clontech) was performed according to the instructions of the manufacturer. The blots containing 2 μg of poly (A)⁺ RNA were hybridized with the following radiolabeled oligonucleotide probes: microsomal GST-II, 5-CAG TGA GAA ACC GAT GGT CCG TTT TTG AGT CTA TCT TTA ACG TAC AGC GCT TCT-3; LTC₄ synthase, 5-TCC GGT ACA GCG TTA GCA GCC TGA GCT GCC C-3. These probes were selected from areas with minimal DNA sequence identity between FLAP, LTC₄ synthase, and microsomal GST-II. For the labeling of the oligonucleotide probes we used [γ-³²P]ATP (DuPont) and poly nucleotide kinase (74 TNK Pharmacia Biotech A/S). Prehybridization/hybridization solutions contained the probes and buffered with a stripping solution (1% SDS) as described by the manufacturer (Clontech) at 47°C. After hybridization and washing, the blots were exposed to x-ray film (Kodak Biomax™ MR) at ~70°C. The exposure time for microsomal GST-II and FLAP was 36 and 72 h, respectively.

RT-PCR—Total RNA was isolated from granulocytes and mononuclear cells using Trizol™ Reagent (Life Technologies, Inc.) according to the instructions of the manufacturer. Subsequently, cDNA was prepared from 2 μg of total RNA in a 40-μl incubation volume using a first strand cDNA synthesis kit obtained from Boehringer Mannheim. PCR was carried out in 100 μl of incubation mixtures consisting of 2 μl of cDNA, 0.2 μl of dNTPs, 0.5 μl each primer, and 2 units of Taq DNA polymerase (Boehringer Mannheim) in PCR buffer (10 mM Tris–HCl, 1.5 mM MgCl₂, 50 mM KCl, pH 8.3). The conditions of the reaction were 1) 94°C for 4 min, 2) 94°C for 45 s, 60°C for 45 s, 72°C for 45 s, 3) 72°C for 10 min; 25 cycles were carried out for both β-actin and microsomal GST-II. Primers for microsomal GST-II were 5-ATT CTC TCG GCC TGT CAG CAA AGT TAT-3 and 5-CAG TGA GAA ACC GAT GGT CCG TTT TTT AGC-3 for regions which were specific for microsomal GST-II as determined from Clustal program (B38-base pair control primer set). The expected size of the microsomal GST-II DNA fragment was 294 base pairs. Aliquots of the PCR mixtures (10 μl) were analyzed by electrophoresis using 1% agarose gels containing 0.5 μg/ml ethidium bromide. The identity of the PCR product corresponding to 294 bp from both granulocytes and mononuclear cells was confirmed by subcloning into pBluescript vector followed by DNA sequencing as described earlier.

Analysis of LTC₄ Synthase Activity in Infected Sf9 Cells—S9 cells were infected with microsomal GST-II, LTC₄ synthase, or mock virus at a density of 1 × 10⁶ cells/ml. 72 h postinfection, the cells were harvested, washed, and pelleted by centrifugation at 300 × g. The cell viability was estimated by trypan blue exclusion. The cells were harvested and protein concentration was determined by the Coomassie protein assay according to the manufacturer’s instructions (Pierce). In order to measure LTC₄ synthase activity, the protein concentration was adjusted to 1 mg/ml using potassium inorganic phosphate buffer (0.1 M, pH 7.4). Then 50 μl of protein was mixed with 50 μl of potassium inorganic phosphate buffer containing 10 mM reduced glutathione and 0.1% (w/v) bovine serum albumin. The reaction was started by the addition of LTC₄ (2 μl of 1.5 mM LTC₄ in ETOH). The reaction was terminated after 15 min by the addition of 100 μl of acetonitrile/methanol:acetic acid (50:50:1, v/v). Precipitated protein was removed by centrifugation at 14,000 × g for 10 min. Subsequently, 150 μl of the sample was analyzed by reverse-phase HPLC equipped with a NovaPack C18 column (3.0 × 150 mm, 4-μm particle size).
Identification of Human Microsomal GST-II

A TBLASTN search of the GenBank™ database using the FLAP peptide sequence revealed that an expressed sequence tag (EST) clone corresponding to this sequence, accession number H59143 displayed significant sequence identity to FLAP (amino acids 51–67), LTC4 synthase, and GST-I. The only significant RNA detected in human tissue blots (Fig. 4, A, B, and G). Microsomal GST-II was also heavily expressed in the adrenals, especially the cortex (Fig. 4E). We also investigated the expression of FLAP and microsomal GST-II in different organs of the immune system and cancer cell lines (Fig. 4, F and G). Microsomal GST-II was expressed in the human promyelocytic leukemia HL60 and HeLa cell lines, but not in the T or B cell lines MolT4 and Raji, respectively. Microsomal GST-II was found also in chronic myelogenous leukemia cell line K-562, adenosarcoma SW480, and melanoma G361. FLAP was expressed in lymph node tissue, thymus, appendix, peripheral blood leukocytes, and bone marrow. FLAP was also detected in HL60 cells and Raji at lower amounts in MolT4 and the adenosarcoma SW480. The expression of microsomal GST-II and FLAP in different brain tissues is shown in Fig. 4D. The only significant RNA detected in brain was FLAP in the medulla and spinal cord. We also investigated the expression of LTC4 synthase using various human tissue blots (Fig. 4, A, D, F, and G); however, utilizing the hybridization described, no detection of LTC4 synthase mRNA was obtained after 3 days of exposure (data not shown). The expression of microsomal GST-II in human peripheral blood leukocytes was analyzed by RT-PCR. Fig. 5 shows that total RNA isolated from mononuclear or polymorphonuclear...
leukocytes contains microsomal GST-II. The identity of the PCR fragment was confirmed by subcloning and full-length sequencing (data not shown).

**Baculovirus Expression of Microsomal GST-II and LTC4 Synthase**—The cDNA insert of clone 204168 microsomal GST-II and the cDNA for LTC4 synthase were subcloned into the pFastBac plasmid followed by creation and isolation of bacmid DNA. Also, a bacmid mock DNA was created. 72 h after transfection of Sf9 cells with bacmid DNA, mRNA from the Sf9 cells was isolated and analyzed by Northern blot and RT-PCR. Microsomal GST-II mRNA was specifically detected using both methods. The corresponding transfection viral stocks were amplified once and subsequently used for infection of Sf9 cells. Following infection, the effects of the viruses on cell viability and growth were observed and compared with noninfected cells.

Leukotriene C4 Synthase Activity—Three 250-ml Sf9 insect cell cultures were infected with recombinant baculoviruses expressing LTC4 synthase, microsomal GST-II, and mock, respectively. A 10-ml aliquot of cells was removed every 24 h, and cells were washed once in PBS, pelleted, and frozen at −80 °C. After 5 days, the cells were thawed and sonicated (crude homogenate). An aliquot was removed to prepare 100,000 × g pellet and supernatant. LTC4 activity was assayed in incubation mixtures containing 0.5 mg/ml sample protein in 0.1 M potassium phosphate (pH 7.4), 0.05% albumin, 5 mM glutathione, and 30 μM LTA4. After 15 min the reaction was terminated by adding 100 μl of stop solution (AcN:MeOH:HAc, 50:50:1), and 150 μl was subjected to RP-HPLC analysis. Table I shows the formation of LTC4 in Sf9 cells after infection. In both noninfected and mock-infected crude homogenates, only very small amounts of LTC4 formation could be detected (<35 pmol/mg protein). However, cells infected with either LTC4 synthase or microsomal GST-II both catalyzed the formation of LTC4 (Table I). The enzymatic activity for LTC4 synthase and microsomal GST-II was 3–5 times higher in the 100,000 × g pellet compared with the activity in the crude homogenates (Table I). The corresponding 100,000 × g cytosol fraction contained no significant activity (data not shown). The microsomal enzymatic activities were 1375 ± 405 pmol LTC4/mg protein/15 min (mean ± S.D., n = 3) for cells infected with LTC4 synthase and 3480 ± 528 pmol LTC4/mg protein/15 min (mean ± S.D., n = 3) for cells infected with microsomal GST-II. The formation of LTC4 was dependent on the presence of both LTA4 and reduced glutathione. Also, the LTC4 formation was abolished by boiling for 5 min prior to the assay. Fig. 6 shows LTC4 formation as a function of time. Microsomal GST-II microsomes were incubated at both 0.5 mg/ml and LTC4 synthase at 0.5 mg/ml led to similar time courses, and the formation of LTC4 was considered linear up to 3 min for microsomal GST-II and up to 5 min for LTC4 synthase (Fig. 6). In order to determine the apparent Kₘ for LTA₄ (at a constant GSH concentration of 5 mM), microsomal GST-II microsomes (0.1 mg/ml) and LTC4 synthase microsomes (0.5 mg/ml) were incubated at various LTA₄ concentrations for 3 and 5 min respectively. Using hyperbolic regression analysis, the apparent Kₘ was 41 μM for microsomal GST-II and 7 μM for LTC4 synthase (Fig. 7). Fig. 8A shows the RP-HPLC chromatograms of the products formed after incubation of LTC4 synthase microsomes (0.5 mg/ml), microsomal GST-II microsomes (0.1 mg/ml) mock virus micro-

**Fig. 2.** Alignment of LTC4 synthase, microsomal GST-II, and FLAP. Residues that are conserved in two of the three proteins are shown in boldface type. The consensus represents amino acids that are conserved in all of the proteins.
somes (0.5 mg/ml), and buffer alone with 30 μM LTA₄ and 5 mM glutathione for 15 min. Peak 2 coelutes with synthetic standard LTC₄ (40 pmol). However, microsomal GST-II also catalyzed the formation of another product (peak 1), eluting as a more polar compound on RP-HPLC. The UV absorbance spectra were compared, and Fig. 8B shows that the spectra corresponding to peak 2 in Fig. 8A all had UV maxima at 281 nm, whereas the spectra corresponding to peak 1 had UV maxima shifted +2 nm. The formation of this product was also dependent on the presence of both LTA₄ and glutathione, abolished by boiling prior to the incubation, time-dependent, and saturable (data not shown).

Glutathione S-Transferase Activity—GST activity was measured spectrophotometrically by measuring formation of the conjugate of the reduced GSH and the CDNB at 340 nm. Microsomes from Sf9 cells expressing microsomal GST-II catalyzed the conjugation of CDNB and GSH at 68 ± 6 nmol/mg/min (mean ± S.D., n = 3). In comparison, the activity found in rat and human liver microsomes has been reported to be 94 and 76 nmol/mg/min, respectively (42). In purified preparation the specific activity for both purified rat and human microsomal GST-I has been reported to be 2 μmol/min/mg (43, 55). The corresponding activities in microsomes from Sf9 cells infected with LTC₄ synthase and mock virus were 11 ± 1 and 10 ± 3 nmol/mg/min, respectively (mean ± S.D., n = 3). In buffer, the rate of nonenzymatic conjugation was 5 ± 2 nmol/mg/min (mean ± S.D., n = 3). Fig. 9 shows the time course of the conjugation of GSH and CDNB by microsomes from Sf9 cells infected with microsomal GST-II, LTC₄ synthase, and mock virus as well as buffer control. Also, the effect of N-ethylmaleimide was investigated. The activity in Sf9 cell microsomes containing microsomal GST-II was not affected by treatment of the protein with 1 mM N-ethylmaleimide.

DISCUSSION

A TBLASTN search of the GenBank™ data base using the FLAP peptide sequence has revealed a new clone with significant sequence identity to FLAP and LTC₄ synthase and with limited sequence identity to microsomal GST. This novel protein retains LTA₄ and 1-chloro-2,4-dinitrobenzene-conjugating activity with reduced glutathione and was termed microsomal glutathione S-transferase II (Fig. 1). Sequence comparison of microsomal GST-II, LTC₄ synthase,
and LTC4 synthase (amino acids 47–63) and FLAP (amino acids 51–67) display a limited sequence identity to microsomal GST-I (amino acids 70–86). Furthermore, as shown in Fig. 3, these regions correspond to the carboxyl terminus of the first hydrophilic stretch in all four proteins. Interestingly, a series of deletion mutants of FLAP in this region (deletions 37–53, 52–58, and 59–61) have demonstrated that this part of FLAP is critical for binding of leukotriene biosynthesis inhibitors such as MK-886 and L-689,037 (56, 57). Also, purified LTC4 synthase has 20,000 times higher specific activity compared with purified human microsomal GST-I to form LTC4 from LTA4 and glutathione (46). In line with the fact that LTC4 synthase and microsomal GST-I both bind LTA4, whereas rat and human microsomal GST-I have been shown to catalyze the reduction of phospholipid hydroperoxides (41), this region is a candidate for being the binding site for the fatty acid backbone component of the various substrates/ligands.

Due to the hydrophobic pattern of FLAP (three hydrophobic regions separated by two hydrophilic regions) it has been proposed that FLAP spans a membrane bilayer 3 times (10). Interestingly, all of these four membrane proteins of approximately the same length display a similar hydrophobicity pattern (Fig. 3). The fact that human microsomal GST-I displays this hydrophobicity pattern plus the limited sequence identity to the other three proteins indicate that they all are members of a family of membrane proteins with highly specialized functions.

Both LTC4 synthase and microsomal GST-I conjugate glutathione with electrophilic substrates. LTC4 synthase has a narrow substrate specificity (37–39) compared with microsomal GST-I (41). Also, purified LTC4 synthase has 20,000 times higher specific activity compared with purified human microsomal GST-I to form LTC4 from LTA4 and glutathione (46). In Table I and Fig. 6, we show that Sf9 cells infected with recombinant baculovirus for microsomal GST-II became capable of catalyzing the formation of LTC4 from LTA4 and reduced glutathione. The specific activity was 3–5 times higher in the 100,000 × g pellet as compared with the total cellular extracts. The microsomal activity was about 5 times higher for microsomal GST-II than LTC4 synthase. This may reflect a better affinity of LTA4 to microsomal GST-II than LTC4 synthase. This pattern may reflect a better expression efficiency for microsomal GST-II and/or a higher Km value for the formation of LTC4. The activity obtained for LTC4 synthase in this expression system was in the same range of activity as reported by others using baculovirus expression (15). The apparent Km values were calculated using hyperbolic regression analysis.

Fig. 6. Time course of formation of LTC4. Microsomes from Sf9 cells expressing microsomal GST-II (0.5 mg/ml and 0.1 mg/ml) or LTC4 synthase (0.5 mg/ml) were incubated with LTA4 (30 μM) and glutathione (5 mM) in the presence of albumin (0.05%) for the indicated times. LTC4 formation was analyzed by RP-HPLC. Also, microsomes (0.5 mg/ml) from Sf9 cells infected with mock virus were included as controls.

Fig. 7. Dependence of the activity of LTC4 synthase (A) and microsomal GST-II (B) on LTA4 concentration. Microsomal preparations of Sf9 cells expressing LTC4 synthase (0.5 mg/ml) and microsomal GST-II (0.1 mg/ml) were incubated with LTA4 (various concentrations) and glutathione (5 mM). The LTC4 synthase reaction was terminated after 5 min, and the microsomal GST-II reaction was terminated after 3 min. Product formation was analyzed by RP-HPLC. The indicated apparent Km values were calculated using hyperbolic regression analysis.
Microsomes from Sf9 cells infected with recombinant baculovirus for microsomal GST-II also catalyzed the conjugation of glutathione and 1-chloro-2,4 dinitrobenzene at a rate of 68 nmol/mg/min. This activity corresponds to 3-4% of the activity reported for purified rat or human microsomal GST-I (43, 55). The rate of conjugation of glutathione and 1-chloro-2,4 dinitrobenzene by LTC4 synthase was similar to that detected in Thera of conjugation of glutathione and 1-chloro-2,4 dinitrobenzene by human GST-I (43, 55) and CDNB (1 mM) for the indicated times. The product formation was monitored by UV absorbance at 340 nm. Also, microsomes (0.125 mg/ml) from Sf9 cells infected with mock virus as well as buffer without microsomes were incubated as controls.

The corresponding Northern blot analysis of LTC4 synthase did not detect any mRNA. This suggests that LTC4 synthase mRNA expression is highly regulated and possibly restricted in blood to various nonabundant leukocytes, in line with the report showing that enriched human eosinophils but not peripheral polymorphonuclear leukocytes express LTC4 synthase (16). Interestingly, the data in Fig. 4 show that microsomal GST-II is highly expressed in the human cell line K-562, a chronic myelogenous leukemia cell line. This evokes the question as to whether or not microsomal GST-II might be the enzyme responsible for the reported increase of LTC4 formation in leukocytes isolated from patients with chronic myelogenous leukemia (32). Also, it will be interesting to investigate the relative influence of LTC4 synthase versus microsomal GST-II on LTC4 formation in cells apparently devoid of 5-lipoxygenase such as platelets, endothelial cells, and smooth muscle cells.

These results indicate that microsomal GST-II is a microsomal protein with both LTC4 synthase activity and the capacity to conjugate CDNB with glutathione. Its catalysis of LTC4 formation seems to be more nonspecific compared with the catalysis per se of glutathione S-transferase; however, its biological function should be further investigated. An intriguing possibility is that FLGST-I may also possess prostaglandin E synthase activity, since a membrane-associated prostaglandin E synthase has been reported as a 17.5-kDa protein requiring glutathione as a cofactor (59, 60). Other possibilities are that this protein represents a general metabolic system for detoxifying fatty acid isomer of LTC4 (data not shown).

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These results indicate that microsomal GST-II is a microsomal protein with both LTC4 synthase activity and the capacity to conjugate CDNB with glutathione. Its catalysis of LTC4 formation seems to be more nonspecific compared with the catalysis performed by LTC4 synthase. Also, its wide tissue distribution resembles microsomal GST-I (45, 58). Consequently, at this stage microsomal GST-II must be categorized as a microsomal glutathione S-transferase; however, its biological function should be further investigated. An intriguing possibility is that FLGST-I may also possess prostaglandin E synthase activity, since a membrane-associated prostaglandin E synthase has been reported as a 17.5-kDa protein requiring glutathione as a cofactor (59, 60). Other possibilities are that this protein represents a general metabolic system for detoxifying fatty acid.
epoxides such as those derived through cytochrome P450 pathways, or as is the case for microsomal GST-I, the protein may possess glutathione peroxidase activity (61).

In summary, we have identified a novel microsomal glutathione S-transferase with significant amino acid identity to FLAP and LTC4 synthase. This enzyme can catalyze the conjugation of both LTA4 and CDNB with glutathione and therefore represents a unique microsomal glutathione S-transferase. The hydrophobicity profile of microsomal GST-II, FLAP, LTC4 synthase, and microsomal GST-I as well as their sequence homologies suggest an evolutionary relationship within this gene family.

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