Production of Leukotriene C\textsubscript{4} in Different Human Tissues Is Attributable to Distinct Membrane Bound Biosynthetic Enzymes*

(Received for publication, November 5, 1996, and in revised form, December 30, 1996)

Kylie A. Scoggan§§, Per-Johan Jakobsson¶, and Anthony W. Ford-Hutchinson||

From the Merck Frosst Centre for Therapeutic Research, Pointe Claire-Dorval, Quebec, Canada H9R 4P8 and the
§§Department of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, Canada H3G 1Y6

Microsomal glutathione S-transferase-II (GST-II) has recently been discovered and characterized as a member of the 5-lipoxygenase-activating protein (FLAP)/5(S)-hydroxy-6(R)-S-glutathionyl-7,9-trans-11,14-cis-eicosatetraenoic acid (LTC\textsubscript{4}) synthase gene family, which also includes microsomal glutathione S-transferase-I (GST-I) as a distant member of this gene family. This new enzyme is unique as it is the only member of this family capable of efficiently conjugating reduced glutathione to both 5,6-oxido-7,9,11,14-eicosatetraenoic acid (LTA\textsubscript{4}) and 1-chloro-2,4-dinitrobenzene. Although microsomal GST-II has been demonstrated to display both general glutathione S-transferase (GST) and specific LTC\textsubscript{4} synthase activities, its biological function remains unknown. In this study, we investigated the physiological location of microsomal GST-II as well as the relative importance of this enzyme versus LTC\textsubscript{4} synthase for the production of LTC\textsubscript{4} in various human tissues and cells that have been previously demonstrated to possess LTC\textsubscript{4} synthase activity. As determined by Western blot, microsomal GST-II was predominately expressed in human liver microsomes, human endothelial cell membranes, and sparsely detected in human lung membranes. In contrast, LTC\textsubscript{4} synthase was prevalent in human lung membranes, human platelet homogenates, and human kidney tissue. Concomitant to the formation of LTC\textsubscript{4}, microsomal GST-II also produces a new metabolite of LTA\textsubscript{4}, a postulated LTC\textsubscript{4} isomer. This isomer was used to distinguish between microsomal GST-II and LTC\textsubscript{4} synthase activities involved in the biosynthesis of LTC\textsubscript{4}. Based on the relative production of LTC\textsubscript{4} to the LTC\textsubscript{4} isomer, microsomal GST-II was demonstrated to be the principal enzyme responsible for LTC\textsubscript{4} production in human liver microsomes and human endothelial cells and played a minor role in the formation of LTC\textsubscript{4} in human lung membranes. In comparison, LTC\textsubscript{4} synthase was the main enzyme capable of catalyzing the conjugation of reduced glutathione to LTA\textsubscript{4} in human lung membranes and human platelet homogenates. Therefore, microsomal GST-II appears to be an integral component in the detoxification of biological systems due to its marked presence in human liver, in accordance with its known GST activity. Microsomal GST-II, however, may also be pivotal for cysteinyll leukotriene formation in endothelial cells, and this could change our current understanding of the regulation of leukotriene biosynthesis in inflammatory disorders such as asthma.

Microsomal glutathione S-transferase (GST)-II\textsuperscript{1} is the newest member of the FLAP/LTC\textsubscript{4} synthase gene family discovered to date. This novel enzyme displays 33% identity to FLAP, 44% identity to LTC\textsubscript{4} synthase, and limited sequence identity to microsomal GST-I at the amino acid level (1). These four proteins may also have similar structural configurations based on analogous hydrophobicity plots. Microsomal GST-II is a 16.6-kDa protein with a calculated pl of 10.4, possesses both LTC\textsubscript{4} synthase as well as conventional GST activities as shown through its ability to conjugate reduced glutathione (GSH) with both LTA\textsubscript{4} and 1-chloro-2,4-dinitrobenzene, and was consequently characterized as a member of the membrane bound GSTs (1). This new enzyme, accordingly, may have important roles in both leukotriene biosynthesis and in cellular detoxification by GST activity.

GSTs are a family of enzymes that catalyze the conjugation of reduced GSH to a variety of electrophilic substrates. The GSTs belong to a gene superfamily in which four different gene families encode the cytosolic GSTs (α, µ, π, and θ) and two encode the microsomal forms of the enzyme (1, 2). Biologically, these enzymes are responsible for detoxification of xenobiotics by catalyzing GSH conjugation to generated metabolites and for protection from endogenous hydroperoxides produced during oxidative stress via their GSH-dependent peroxidase activity (3).

LTC\textsubscript{4} synthase is a unique membrane bound enzyme that has been distinguished from all previously known cytosolic and microsomal GSTs (4, 5) through its narrow substrate specificity for LTA\textsubscript{4}. LTC\textsubscript{4} synthase and FLAP are important components of the leukotriene biosynthetic pathway. FLAP is required for cellular LTA\textsubscript{4} formation, possibly through the presentation of arachidonate to 5-lipoxygenase (6–9). LTC\textsubscript{4} synthase is the first committed enzyme for the conversion of LTA\textsubscript{4} to the cysteinyll leukotrienes, LTC\textsubscript{4}, LTD\textsubscript{4}, and LTE\textsubscript{4}, which have significant roles in immediate hypersensitivity reactions (see reviews in Refs. 10–15).

* This work was supported in part by grants from The Wenner-Gren Foundation, The Heart and Lung Foundation, The Helmhuth Hertz Foundation, the Swedish Society of Medicine, Ulla and Gustaf af Ugglas’s Foundation, and The Swedish Foundation for International Cooperation in Research and higher education (to P-J. J.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§§Recipient of the Canadian Medical Research Council Industrial Studentship Award.
¶Recipient of a Postdoctoral Fellowship from the Karolinska Institute.
||To whom correspondence should be addressed: Vice-President of Research, Merck Frosst Centre for Therapeutic Research, P.O. Box 1005, Pointe Claire-Dorval, Quebec, Canada H9R 4P8. Tel.: 514 428-2620; Fax: 514 428-2624.

1 The abbreviations used are: GST, glutathione S-transferase; FLAP, 5-lipoxygenase activating protein; LTA\textsubscript{4}, 5,6-oxido-7,9,11,14-eicosatetraenoic acid; LTC\textsubscript{4}, 5(S)-hydroxy-6(R)-S-glutathionyl-7,9-trans-11,14-cis-eicosatetraenoic acid; LTD\textsubscript{4}, 5(S)-hydroxy-6(R)-S-cysteinylglycyl-7,9-trans-11,14-cis-eicosatetraenoic acid; LTE\textsubscript{4}, 5(S)-hydroxy-6(R)-S-cysteinyl-7,9-trans-11,14-cis-eicosatetraenoic acid; GSH, glutathione; PMHS, phenylmethylsulfonyl fluoride; HPLC, high performance liquid chromatography; PBS, phosphate-buffered saline; T-TBS, Tris-buffered saline containing Tween 20.
Microsomal GST-II versus LTC₄ Synthase in LTC₄ Production

Prior to the molecular characterization of LTC₄ synthase and microsomal GST-II, a number of studies have described LTC₄ synthase activity in various cell types and tissues (15–24). The present study addresses the relative importance of these two enzymes in the synthesis of LTC₄ in various human tissues.

**EXPERIMENTAL PROCEDURES**

**Materials**—Human venous endothelial frozen cell pellet was purchased from Cell Systems (Kirkland, WA). Human liver and lung tissues were obtained from the International Institute for the Advancement of Medicine (IIAM) (Exton, PA). Protein medleys were from Clontech (Palo Alto, CA). Bovine serum albumin, phenylmethylsulfonyl fluoride (PMSF), Hepes, and dithiothreitol were purchased from Sigma. Aprotinin, leupeptin, and pepstatin A were from Boehringer Mannheim GmbH, (Mannheim, Germany). Tris base was also from Boehringer Mannheim Corporation (Indianapolis, IN). Taurocholic acid, sodium salt was obtained from Calbiochem Corporation (La Jolla, CA). LTA₂, methyl ester, 2-(2-(1-(4-chlorobenzyl)-4-ethylphenyl)ethyl)thiopyrano(2,3,4-H)-thiopyrano(2,3,4-H)-Frosst Centre for Therapeutic Research. Novapak C18 HPLC columns (Waters Corp., Milford, MA) were purchased from Waters Associates. Bovine serum albumin, phenylmethylsulfonyl fluoride (PMSF), Hepes, and dithiothreitol were purchased from Sigma.

**Preparation of Human Endothelial Cell, Liver, and Lung Membranes**—A frozen human venous endothelial cell pellet containing 1 × 10⁶ cells was thawed on ice and resuspended in PBS containing 2 mM EDTA and 2 mM PMSF. The cells were sonicated three times for 15 s each on ice and subjected to differential centrifugation at 1,000 × g for 10 min and 100,000 × g for 1 h, both at 4 °C. The pellets were resuspended in 50 µl of PBS containing 2 mM EDTA, frozen in liquid nitrogen, and stored at −80 °C. Bradford (28) protein assay (Bio-Rad) was performed on the homogenate.

**Preparation of Human Endothelial Cell, Liver, and Lung Membranes**—A frozen human venous endothelial cell pellet containing 1 × 10⁶ cells was thawed on ice and resuspended in PBS containing 2 mM EDTA and 2 mM PMSF. The cells were sonicated three times for 15 s each on ice and subjected to differential centrifugation at 1,000 × g for 15 min and 100,000 × g for 1 h, both at 4 °C. The pellets were resuspended in 50 µl of PBS containing 2 mM EDTA, frozen in liquid nitrogen, and stored at −80 °C. Subcellular fractions of frozen human liver and lung tissues were prepared according to standard procedures (see Refs. 29 and 30, respectively).

**Western Blot Analyses of Microsomal GST-I, Microsomal GST-II, LTC₄ Synthase, and FLAP Expression**—Western blot analyses were performed similarly to those described previously (26). Briefly, SDS-polyacrylamide gels (Novex), and electrophoresed onto nitrocellulose. Ponceau S staining was used to visualize the efficiency of transfer. Membranes were then soaked for 1 h at 25 °C in Tris-buffered saline containing 0.1% (v/v) Tween 20 (0.1% T-TBS) (20 mM Tris/HCl (pH 7.5), 0.5 M NaCl), containing 5% (v/v) Bio-Rad blotting grade non-fat dry milk. Blots were washed twice for 5 min each with 0.1% T-TBS and subsequently treated for 1 h at 25 °C with the indicated specific primary polyclonal antibody (dilution 1:500) in 0.05% T-TBS containing 5% dry milk. After washing the blots 3 times for 5 min each with 0.1% T-TBS, the membranes were incubated for 1 h at 25 °C with a horseradish-peroxidase-linked donkey anti-rabbit antibody (dilution 1:3,000) in 0.05% T-TBS containing 5% dry milk. The blots were washed 3 times for 5 min each with 0.1% T-TBS and subsequently developed using enhanced chemiluminescence (Renaissance Western blot chemiluminescence reagent, DuPont NEN) according to the manufacturer instructions.

**Measurement of LTC₄ Synthase and Microsomal GST-II Enzymatic Activities by Reverse-phase HPLC**—LTC₄ synthase and microsomal GST-II activities were assayed as described previously (1) by measuring the amount of LTC₄ and an isomer of LTC₄ produced in incubations at 25 °C from various samples in 0.1 M potassium phosphate buffer (pH 7.4) containing reduced glutathione (5 mM) and 60 µM LTα₁ (free acid) stabilized by the presence of 0.05% (w/v) bovine serum albumin in a final volume of 100 µl. After 15 min, the reaction was terminated by the addition of an equimolar amount of acetylimidazole/methanol/acetic acid at 50:50:1, and the precipitated proteins were removed by centrifugation at 16,000 × g for 15 min at 4 °C. The amount of LTC₄, and an isomer of LTC₄ synthesized were resolved by isocratic reverse-phase HPLC on a Waters Associates Novapak C₁₈ column (3.0 × 150 mm, 4 µm particle size) with the mobile phase (acetonitrile/methanol/water/acetic acid at 28:14:54:1 (pH 6.5)) at a flow rate of 1.2 ml/min. Quantification of the amount of products formed was based on the measurement of the peak absorbance at 280 nm from known amounts of injected LTC₄, LTC₂, and the LTC₂ isomer peaks were identified by comparison to retention time of synthetic LTC₄ and on-line analysis of the Waters 991 diode-array spectrophotometer.

**Preparation of Microsomal GST-II, LTC₄ Synthase, and FLAP Membranes from Baculovirus-infected Sf9 Cells**—Microsomal GST-II, LTC₄ synthase, and FLAP proteins were obtained from baculovirus-infected Sf9 cell membranes as described previously (1, 8). Briefly, Sf9 cells (Invitrogen) were infected with recombinant or wild-type virus and cultured for 72 h at 28 °C in Grace’s insect media (Life Technologies, Inc.) supplemented with 10% fetal bovine serum (v/v), gentamycin (50 µg/ml), and fungizone (25 µg/ml). The cells were then harvested, washed, resuspended in PBS, and sonicated three times for 10 s each on ice. The sonicates were subsequently subjected to differential centrifugation at 500 × g for 10 min and 100,000 × g for 1 h, both at 4 °C. The pellets were resuspended in PBS and stored at −80 °C. Coomassie protein (Pierce) assay was performed according to the manufacturer instructions.

**RESULTS**

**Microsomal GST-I, microsomal GST-II, and FLAP polyclonal antibodies are specific, whereas, the LTC₄ synthase antibody displays cross-reactivity**—Polyclonal antibodies were used as tools to determine the relative contribution of LTC₄ synthase versus microsomal GST-II for the production of LTC₄ in biological systems. To determine the presence of either of these proteins, the specificity of these antibodies with respect to one another and to other members of this family, FLAP and microsomal GST-I, were tested. The antibodies used to detect microsomal GST-II, LTC₄ synthase, and FLAP had been raised against peptides in the region displaying the highest identity to one another, the FEB region (Fig. 1). The microsomal GST-I polyclonal antibody was raised against the full-length protein, which also contains a similar region of homology to the other peptides. As the different peptides contain homologous regions, the four antibodies were tested for possible cross-reactivity by Western blot analyses. Fig. 2 demonstrates that microsomal GST-I, microsomal GST-II, and FLAP antibodies were specific. However, the LTC₄ synthase antibody displayed some cross-reactivity by detecting microsomal GST-II and FLAP proteins. These results indicate that we have
specific polyclonal antibodies for the detection of microsomal GST-I, microsomal GST-II, and FLAP and a polyclonal antibody that cannot distinguish between LTC4 synthase and microsomal GST-II.

Expression of Microsomal GST-I, Microsomal GST-II, LTC4 Synthase, and FLAP in Various Human Tissues—After establishing the specificity of the above polyclonal antibodies, they were subsequently used to search for their respective protein targets in various human tissues (Fig. 3). As expected, microsomal GST-II was markedly detected in liver tissue, as well as in adrenal gland, kidney, and to a lesser extent mammary gland tissues. Microsomal GST-II was highly expressed in liver (Fig. 3) and in lower amounts in adrenal gland, kidney, pancreas, and thymus tissues (after prolonged exposure, data not shown). The LTC4 synthase antibody detected 17-kDa proteins intensely in kidney, adrenal gland, liver, and lymph and weakly in skeletal and stomach samples, whereas, the FLAP antibody displayed strong 18-kDa bands in lymph and thymus tissues and weak bands in adrenal gland, liver, and skeletal tissues. These results have identified many important human tissues in which these proteins may be found.

Expression of LTC4 Producing Enzymes in Human Liver, Lung, Endothelial Cells, and Platelets—Fig. 3 demonstrates that microsomal GST-II was primarily present in human liver tissue. To confirm and extend this finding, the subcellular localization of microsomal GST-II was investigated in human liver tissue by probing liver samples prepared by differential centrifugation with the microsomal GST-II and LTC4 synthase polyclonal antibodies (Fig. 4). Both microsomal GST-II and LTC4 synthase antibodies displayed an 17-kDa band in the liver homogenate, the 10,000 × g supernatant, and the
Microsomal GST-II versus LTC₄ Synthase in LTC₄ Production

**DISCUSSION**

Recently, a novel membrane bound protein, microsomal GST-II, was discovered that displayed homology at the amino acid level to FLAP, LTC₄ synthase, and to a lesser extent microsomal GST-I (1). These proteins all display similar hydrophobicity patterns and consequently may have similar structures. Due to the above similarities, these four proteins appear to be members of a gene family that encodes membrane bound proteins important for either leukotriene production or cellular detoxification by GSH conjugation. Microsomal GST-II is a unique member of this family due to its ability to efficiently conjugate reduced GSH to both LTA₄ and to 1-chloro-2,4-dinitrobenzene (1). Hence, microsomal GST-II may be more important than LTC₄ synthase as a catalyst for the formation of LTC₄ in certain tissues. To try to understand the biological function (importance) of this unique enzyme, its location in human cells and tissues compared with other family members was determined by Western blot analyses. In addition, a new method was developed for distinguishing between previously determined LTC₄ synthase activity and what may actually be microsomal GST-II activity.

Microsomal GST-I, microsomal GST-II, and FLAP polyclonal antibodies were determined to be specific for the recognition of their respective proteins even though the peptides used to raise

---

**FIG. 4.** Human liver and endothelial cell membranes principally express microsomal GST-II, while, human lung and platelet homogenates primarily express LTC₄ synthase. Differential centrifugation fractions from human liver (75 µg of protein), human lung membrane fractions (75 µg of protein), human endothelial cell membranes (10 µl of 1 × 10⁷ cell equivalents), human platelet homogenates (75 µg of protein), microsomal GST-II from baculovirus-infected Sf9 cell membranes (5 µg of protein), and partially purified LTC₄ synthase from THP-1 cell extracts (7.35 µg of protein) were electrophoresed through polyacrylamide gels, electrodotted onto nitrocellulose, and immunoblotted using the polyclonal antibodies to microsomal GST-II or LTC₄ synthase and detected using enhanced chemiluminescence as described under “Experimental Procedures.” The liver, lung, and control blots were exposed to film for 15 s, whereas, the endothelial and platelet blots had an exposure time of 1 min. α-MGST-II, α-microsomal GST-II.

---

**FIG. 5.** Comparison of LTA₄ metabolites produced by microsomal GST-II, versus LTC₄ synthase in human liver, lung, and platelets. This reverse-phase HPLC chromatogram demonstrates the representative LTA₄ metabolites obtained in Table I due to the presence of either microsomal GST-II activity or LTC₄ synthase activity. The scale of the different chromatograms were in all cases normalized to the LTC₄ peak. The LTC₄ peak displayed a retention time of 9.0 min and a maximum UV absorbance at 281 nm, while the postulated LTC₄ isomer peak had a retention time of 7.6 min and a maximum UV absorbance at 283 nm.
Differential centrifugation fractions from human liver (0.4 mg of protein), human endothelial 100,000 × g membrane fractions (0.4 mg of protein), human lung (100,000 × g) membrane fractions (0.4 mg of protein), human platelet homogenates (0.2 mg of protein), partially purified LTC4 synthase from THP-1 cell extracts (2.45 μg of protein), microsomal GST-II from baculovirus-infected Sf9 cell membranes (0.05 mg of protein), LTC4 synthase from baculovirus-infected Sf9 cell membranes (0.05 mg of protein) were prepared as described under “Experimental Procedures.” Microsomal GST-II or LTC4 synthase activity was assayed in the above samples by measuring the amount of LTC4 and a postulated LTC4 isomer produced in 15 min-incubations at 25 °C in 0.1 M KPi, pH 7.4, containing reduced glutathione (5 mM) and 60 μM LTA4 (free acid) stabilized by the presence of 0.05% (w/v) bovine serum albumin, as resolved by isocratic reverse-phase HPLC. The relative production of LTC4 to the postulated LTC4 isomer was used to define microsomal GST-II activity versus LTC4 synthase activity. The liver 100,000 × g pellet samples represent different human livers (n = 6), including one sample representing the mean of a triplicate. The endothelial 100,000 × g membrane fraction is a representative experiment of n = 2. All other samples were performed in triplicate ± S.E.

### Table I

Microsomal GST-II versus LTC4 Synthase in LTC4 Production

| Sample                                | LTC4 formed | Isomer formed | Ratio (LTC4:isomer) |
|---------------------------------------|-------------|---------------|---------------------|
| Liver 100,000 × g sup                 | <50         | –             | –                   |
| Liver 100,000 × g pellet              | 833 ± 147   | 199 ± 25      | <5                  |
| Endothelial 100,000 × g pellet        | 200         | 64            | >5                  |
| Lung 100,000 × g pellet               | 5270 ± 146  | 105 ± 3.5     | >5                  |
| Platelet homogenate                   | 507 ± 10    | –             | >5                  |
| LTC4 synthase/THP-1 partially purified| 129,000 ± 1880 | –             | >5                  |
| LTC4 synthase/Sf9 100,000 × g pellet  | 1280 ± 365  | –             | >5                  |
| MGST-II/Sf9 100,000 × g pellet        | 5430 ± 29   | 2170 ± 16     | <5                  |
| wt Bac/Sf9 100,000 × g pellet         | –           | –             | –                   |

* At the limit of detection, define limit as 15 pmol in assay.
** Ratios not performed due to limit of detection of both LTC4 and the LTC4 isomer.

These antibodies contained regions of homology. Only the LTC4 synthase antibody displayed cross-reactivity by recognizing FLAP, microsomal GST-II, and LTC4 synthase. This nonspecific association of the LTC4 synthase antibody to FLAP may be explained by a combination of high antibody concentration (1:50 dilution) and high expression of FLAP from baculovirus-infected Sf9 cells resulting in its high abundance on the blot. The recognition of FLAP by the LTC4 synthase antibody could be discerned from LTC4 synthase and microsomal GST-II due to the higher molecular weight of FLAP and was therefore not a problem in determining the presence of FLAP in various tissues. This antibody, however, could not discriminate between LTC4 synthase and microsomal GST-II, thus, the presence of LTC4 synthase was inferred based on the absence of specific microsomal GST-II recognition by the microsomal GST-II polyclonal antibody in identical tissues.

Interestingly, microsomal GST-II expressed from Sf9 cells was detected as a doublet, which may indicate the phosphorylation of this enzyme or a downstream degradation product. Phosphorylation of microsomal GST-II could be a possible regulatory mechanism for this enzyme as it has also been postulated for LTC4 synthase (27). LTC4 synthase has two potential protein kinase C phosphorylation sites which are not, however, present in microsomal GST-II. Microsomal GST-II from Sf9 cells was also inhibited by leukotriene biosynthesis inhibitors with a similar IC50 value as that for LTC4 synthase (32). As these inhibitors have been shown to inhibit FLAP and LTC4 synthase, these proteins may have a similar active site. FLAP amino acids in the region Ser31 to Val36 have been shown to be critical for binding of leukotriene biosynthesis inhibitors (33). This region is the most homologous region between the members of this family including microsomal GST-I and is, therefore, postulated to be the lipid binding site of these proteins. As expected, human microsomal GST-I is mainly expressed in human liver tissue, in accordance with previous findings of location and its function as a phase II detoxifying enzyme (3). The significant detection of microsomal GST-I in the adrenal gland and kidney substantiates previous findings of microsomal GST activity, protein expression, or mRNA expression and probably serves to protect these extrapulmonary tissues from endogenous or exogenous toxicants (34, 35). Microsomal GST-II is also primarily expressed in human liver membranes and to a lesser extent in endothelial cells and minimally in lung membranes, displaying a very narrow tissue distribution. The marked detection of microsomal GST-II in the liver along with its glutathione S-transferase activity suggests that it may have a similar biological function to microsomal GST-I. Microsomal GST-II also appears to be post-transcriptionally regulated since the protein was not significantly detected in many of the tissues that exhibit mRNA expression (1). However, this lack of protein detection may also be a matter of sensitivity in the Western blot analyses. FLAP and LTC4 synthase were widely distributed with expression in overlapping tissues as might be anticipated for two proteins involved in the biosynthesis of eicosanoids. However, there were also tissues (kidney, stomach, and thymus) that only expressed either FLAP or LTC4 synthase. Consequently, these tissues demonstrate either the requirement of transcellular metabolism for leukotriene biosynthesis or in the case of those tissues that only express FLAP, may solely produce LTA4. These observations may also indicate the recognition of other putative members of this gene family by the cross-reactive LTC4 synthase antibody. Contamination of tissue preparations by infiltrating peripheral blood cells could also explain any discrepancies in tissue distributions. FLAP was distinctly found in human lymph and thymus tissues, confirming previous findings of FLAP in B- and T-lymphocytes (36), whereas, LTC4 synthase is significantly detected in human kidney, lung, platelets, and to a lesser extent in skeletal tissue, identical to earlier observations of mouse LTC4 synthase mRNA expression (37) and human LTC4 synthase protein detection (38). The LTC4 synthase antibody appears to detect a 17-kDa band in the liver with an equivalent intensity to that detected by the microsomal GST-II antibody. These band intensities are most likely saturated and have reached a plateau due to overexposure of the blots. Therefore, the band intensities displayed in the liver by different antibodies cannot be quantitatively compared.

Microsomal GST-II possesses both generalized GST and LTC4 synthase activities thereby contributing to the formation of LTC4 in biological systems. Microsomal GST-II concomitantly produces LTC4 and a new LTA4 metabolite, a possible isomer of LTC4, that can be separated from LTC4 by reverse-phase HPLC. LTC4 synthase stereoselectively produces LTC4, thus, the production ratio of LTC4 to the LTC4 isomer clearly...
differentiates microsomal GST-II activity versus LTC_{4} synthase activity. Identification of microsomal GST-II activity corresponds directly to the detection of microsomal GST-II in Western blot analyses. There is significant microsomal GST-II activity in liver 100,000 x g membrane samples. Microsomal GST-II is accordingly the predominant membrane bound enzyme responsible for LTC_{4} formation in human liver and may subsequently be a fundamental enzyme responsible for detoxification of lipid epoxides. Based on the above data, however, the possibility that LTC_{4} synthase still exists in the liver in an inactive form cannot be excluded.

LTC_{4} was selectively synthesized by lung membranes with a minor production of the LTC_{4} isomer, demonstrating LTC_{4} synthase as the favored enzyme for LTC_{4} production in human lung tissue with a minimal contribution from microsomal GST-II. In the lung, microsomal GST-II may play a minor part in LTC_{4} production, however, it may have another function such as protection from oxidative stress and inhibited xenobiotics. The limited formation of LTC_{4} from liver cytosol most likely represents the ability of cytosolic GSTs (mostly \mu family GSTs) to conjugate reduced GSH to LTA_{4} (39).

Platelet homogenates produced significant amounts of LTC_{4} in the absence of notable quantities of the LTC_{4} isomer, which together with the Western blot data indicate that the LTC_{4} synthase enzyme predominates for LTC_{4} production in platelets. In contrast, microsomal GST-II was detected in endothelial cell membranes by Western blot and activity assays and may be the enzyme responsible for converting exogenous LTA_{4} to LTC_{4} during transcellular metabolism in these cells. The presence of microsomal GST-II as the main enzyme for LTC_{4} production in endothelial cells could explain the findings of Habib and Maclouf (40). In these studies, endothelial cells and platelets were both demonstrated to produce LTC_{4} from exogenously added LTA_{4}, however, platelets were more efficient at this conversion. Endothelial cells displayed a higher apparent \( K_m \) for LTA_{4} in comparison to platelets. Similarly, microsomal GST-II has a higher \( K_m \) for LTA_{4} than LTC_{4} synthase and is less stereoselective for the production of LTC_{4} (1). Analogous to our results, the HPLC chromatogram demonstrating LTC_{4} production by endothelial cells (40) also contains an extra peak where the LTC_{4} isomer would be predicted to appear. Such a peak is absent or less pronounced in the chromatogram from platelets. These observations may support the present findings, that LTC_{4} production is catalyzed by microsomal GST-II in endothelial cells and by LTC_{4} synthase in platelets.

Overall, microsomal GST-II is an integral membrane bound protein in human liver tissue that is potentially involved in detoxification of biological systems. Importantly, we have developed a method to distinguish microsomal GST-II activity from LTC_{4} synthase activity. This has led to the discovery that microsomal GST-II is predominantly responsible for LTC_{4} production in liver membranes and may be a key enzyme in the transcellular metabolism of LTA_{4} into cysteinyl leukotrienes in certain cells that lack all of the enzymes required for de novo leukotriene biosynthesis.

Acknowledgments—The authors thank Dr. Don Nicholson for help throughout these studies. We also wish to thank Nathalie Chauret and Dr. Deborah Nicoll-Griffith for preparing the human liver subcellular fractions, Dita Rasper for preparing the human lung membranes, and Joseph Mancini for helpful discussions and for supplying the membranes from SB cells expressing FLAP.

REFERENCES

1. Jakobsson, P. J., Mancini, J. A., and Ford-Hutchinson, A. W. (1996) J. Biol. Chem. 271, 22203–22210
2. Rushmore, T. H., and Pickett, C. B. (1993) J. Biol. Chem. 268, 11475–11478
3. Daniel, V. (1993) Anal. Biochem. 215, 1–12
4. MacGlashan, D. W. J., Schleimer, R. P., Peters, S. P., Schulman, E. S., Adams, G. K., Newball, H. H., and Lichtenstein, L. M. (1992) Clin. Invest. 70, 747–751
5. Vickers, P. J., Adam, M., Charleson, S., Coppolino, M. G., Evans, J. F., and Mancini, J. A. (1994) Mol. Pharmacol. 45, 4538–4547
6. Canepa, S. J., and Cannon, P. J. (1986) J. Biol. Chem. 261, 16466–16472
7. Price, L. A., and Evans, J. F. (1990) Anal. Biochem. 182, 256–260
8. Scoggan, K. A., Nicholson, D. W., and Ford-Hutchinson, A. W. (1996) Biochem. Biophys. Acta. 128, 265–270
9. Habib, A., and Maclouf, J. J. (1992) Arch. Biochem. Biophys. 298, 544–552

Downloaded from http://www.jbc.org/ by guest on July 25, 2018
Production of Leukotriene C₄ in Different Human Tissues Is Attributable to Distinct Membrane Bound Biosynthetic Enzymes

Kylie A. Scoggan, Per-Johan Jakobsson and Anthony W. Ford-Hutchinson

J. Biol. Chem. 1997, 272:10182-10187.
doi: 10.1074/jbc.272.15.10182

Access the most updated version of this article at http://www.jbc.org/content/272/15/10182

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 40 references, 12 of which can be accessed free at http://www.jbc.org/content/272/15/10182.full.html#ref-list-1