Biochemical Pharmacology, Vol. 44, No. 10, pp. 2047–2053, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

GLUTATHIONE *S*-TRANSFERASE-DEPENDENT CONJUGATION OF LEUKOTRIENE A_4-METHYL ESTER TO LEUKOTRIENE C_4-METHYL ESTER IN MAMMALIAN SKIN

RAJESH AGARWAL, HAIDER RAZA,* DAVID L. ALLYN, DAVID R. BICKERS and HASAN MUKHTAR†

Department of Dermatology, University Hospitals of Cleveland, Skin Diseases Research Center, Case Western Reserve University, and the Department of Veterans Affairs Medical Center, Cleveland, OH 44106, U.S.A.

(*Received* 12 *February* 1992; *accepted* 24 *July* 1992)

Abstract—The glutathione *S*-transferase (GST)-dependent conjugation of reduced glutathione (GSH) with leukotriene A_4 (LTA_4)-methyl ester in rodent and human skin was investigated. Incubation of [^{3}H]LTA_4-methyl ester (1 nmole, ≈200,000 dpm) with cytosol prepared from rat, mouse and human skin or with affinity purified GST from rat skin cytosol in the presence of GSH resulted in the formation of LTC_4-methyl ester. Maximum enzyme activity was observed in rat skin followed by mouse and human skin. With heat-denatured cytosol or in the absence of GSH, the product formation was negligible. GST purified from rat skin cytosol by GSH-agarose affinity chromatography exhibited a several-fold increase in the specific activity of enzyme with 1-chloro-2,4-dinitrobenzene (55-fold), ethacrynic acid (67-fold) and LTA_4-methyl ester (12-fold) as substrates. Western blot analysis of the affinity purified GST indicated a predominant expression of the Pi class of GST isozyme followed by Mu and Alpha classes of isozymes. The formation of LTC_4-methyl ester was established by its radioactivity profile on high pressure liquid chromatography and absorption spectroscopy. These results suggest that, in addition to xenobiotic metabolism, cutaneous GSTs may also be capable of metabolizing physiological substrates such as LTA_4.

Leukotrienes constitute a group of oxygenated metabolites of arachidonic acid with biological activity related to hypersensitivity and inflammation [1, 2]. Biosynthesis of these compounds proceeds via the unstable intermediate leukotriene A_4 (LTA_4‡) formed by the 5-lipoxygenase pathway of arachidonic acid metabolism. One pathway of LTA_4 biotransformation is its conversion to LTB_4 by LTA_4 hydrolase [3–5]. A major route of LTA_4 biotransformation is conjugation of its epoxy group with reduced glutathione (GSH) leading to the formation of LTC_4 which is then further metabolized to LTD_4 by γ-glutamyltransferase; LTD_4 is metabolized to LTE_4 by dipeptidase [5–7]. These cysteine-containing leukotrienes are biologically active compounds, the slow-reacting substances of anaphylaxis, and are presumed to be mediators of allergic and anaphylactic reactions [5]. The formation of LTC_4 has been demonstrated in mouse myocytoma cells, rat basophilic leukemia cells, rat mononuclear cells, and in human and guinea pig lungs [8–10].

The biotransformation of LTA_4 to LTC_4 catalyzed by cytosolic glutathione *S*-transferase (GST) (EC 2.5.1.18) has been studied in recent years in certain extracutaneous tissues from rats and humans [11–13]. Chang *et al.* [14, 15] have shown that among the rat liver GST isozymes, the anionic isozyme, a homodimer of the Yb subunit (a member of the mu class of GST isozymes), exhibits the highest specific activity towards the conversion of LTA_4 to LTC_4. In general, GST isozymes containing the Yb subunit exhibit greater activity than do isozymes containing the Ya and/or Yc subunits (Alpha class of GST isozymes). It has been shown that purified GSTs from lung and seminal vesicles show little activity towards leukotriene epoxides indicating a lack of the counterpart of the rat liver anionic GST isozyme in these tissues [14, 15].

In spite of the fact that the skin is a major site of inflammation and is susceptible to various allergic and anaphylactic reactions mediated by the cysteine-containing leukotrienes, the ability of GSTs to catalyze the conjugation of LTA_4 to LTC_4 in this tissue has not been investigated. Recently, we have shown the existence of multiple isozymes of GSTs in human and rodent skin [16]. In the present study we purified GST from rat skin cytosol by GSH-agarose affinity chromatography and showed that this preparation effectively mediated the conjugation of GSH with LTA_4-methyl ester resulting in the formation of LTC_4-methyl ester.

MATERIALS AND METHODS

Chemicals. [14,15-$^3H_2$]LTA_4-methyl ester (sp. act.

* Present address: Department of Biochemistry, Faculty of Medicine and Health Sciences, United Arab Emirates University, Al Ain, U.A.E.

† Corresponding author: Hasan Mukhtar, Ph.D., Department of Veterans Affairs Medical Center, 10701 East Boulevard, Cleveland, OH 44106, U.S.A.

‡ Abbreviations: CDNB, 1-chloro-2,4-dinitrobenzene; GSH, reduced glutathione; GST, glutathione *S*-transferase; LTA_4, LTC_4, LTD_4, and LTE_4, leukotriene A_4, C_4, D_4, and E_4, respectively; and SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

42 Ci/mmol) was obtained from New England Nuclear (Boston, MA), and unlabeled LTA_4-methyl ester and LTC_4 were purchased from Biomol Research Laboratories Inc. (Plymouth, PA). Epoxy-activated GSH-agarose and GSH were obtained from the Sigma Chemical Co. (St. Louis, MO). Acrylamide, bisacrylamide and other reagents for electrophoresis were purchased from Bio-Rad (Richmond, CA). Nitrocellulose membrane for Western blot analysis was obtained from the Schleicher & Schuell Corp. (Keene, NH). Reagents for immunoblotting and color development were purchased as a kit from the Promega Biotech Corp. (Madison, WI). Solvents for HPLC and all other chemicals used were obtained in the purest form commercially available.

Animals and preparation of cytosol. Sperm-positive pregnant Sprague–Dawley rats during the last trimester were obtained from the Holtzman Rat Farm (Madison, WI). Neonatal rats born *in situ* were allowed to suckle until day 4 after birth after which they were withdrawn from their mothers and used in the present study. SENCAR mice were obtained from the National Cancer Institute Frederick Cancer Research Facility (Bethesda, MD). The dorsal skin (2 in^2) of the animals was shaved and remaining hairs were removed by the application of the depilatory cream Nair®. Excess Nair® was washed off with running water. The animals were killed by cervical dislocation, and the skin was excised and scraped to remove dermal fat. The skin was then homogenized in 4 vol. of 100 mM phosphate buffer, pH 7.4, containing 150 mM KCl, and the 100,000 *g* supernatant (cytosol) fraction was prepared as described earlier [17]. Human skin samples were obtained from elective abdominoplasties or breast reduction patients from the core facility at the Institute of Pathology, Case Western Reserve University. These specimens were collected within 2 hr of the surgery and were normal by morphological criteria. Skin was dissected free from the adipose tissue and homogenized to prepare the 100,000 *g* supernatant fraction as described earlier [17].

Partial purification of GSTs from rat skin. The usefulness of the neonatal rat as an experimental model for studies on cutaneous xenobiotic metabolism has been described earlier [18]. Cytosolic fraction prepared from whole skin of neonatal rats was collected by the use of a syringe in order to avoid the adherent lipid layer. The material thus obtained was filtered through glass wool to remove any floating lipid material, and the filtrate was used as the enzyme source for further purification. Solid ammonium sulfate was added gradually to cytosol (total volume 200 mL, 4 mg protein/mL) to achieve 80% saturation. The suspension was stirred overnight and centrifuged at 20,000 *g* for 30 min. The pellet was resuspended in 50 mL of 10 mM potassium phosphate buffer, pH 7.2, containing 1 mM EDTA and 1 mM 2-mercaptoethanol (buffer A), dialyzed overnight against 2 L of buffer A (four changes) and centrifuged at 20,000 *g* for 30 min. The supernatant was then subjected to affinity chromatography on GSH-coupled epoxy-activated agarose according to the method of Simons and VanderJagt [19] as described by Singh *et al.* [20]. Briefly the dialyzed, ammonium sulfate cut supernatant was applied to a GSH-agarose column (1.0 × 8.0 cm) pre-equilibrated with 22 mM potassium phosphate buffer, pH 7.2. The column was washed with several volumes of equilibration buffer at a flow rate of 15 mL/hr, and the enzyme was eluted with 50 mM Tris–HCl buffer, pH 9.6, containing 10 mM GSH. The eluted fractions were assayed for GST activity using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate according to the method of Habig *et al.* [21]. The enzyme rich fractions were pooled, dialyzed against 10 mM potassium phosphate buffer, pH 7.4, and concentrated by ultrafiltration using a PM-10 membrane. This preparation is defined as the affinity purified GST in the present study.

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) and Western blot analysis. The affinity purified GST and crude cytosolic preparation from rat skin were subjected to SDS-PAGE and Western blot analysis as described by Laemmli [22], and Towbin *et al.* [23], respectively. The antibodies to Alpha, Mu and Pi classes of GST isozymes, used to probe the Western blots, were gifts from Professor Y. C. Awasthi, Galveston, TX.

Enzyme assay and protein determination. GST activity towards CDNB and ethacrynic acid was determined according to Habig *et al.* [21]. Protein was estimated according to the method of Bradford [24].

Conjugation of LTA_4-methyl ester to LTC_4-methyl ester. A stock solution of [^{3}H]LTA_4-methyl ester was prepared in hexane containing 1% triethylamine by mixing [14,15-$^3H_2$]LTA_4-methyl ester and LTA_4-methyl ester to a concentration of 240 μM with specific activity of 83 Ci/mol, and was stored in small aliquots at −70°. At the time of use in the assay system, hexane was evaporated under a stream of N_2 and an equal volume of methanol was added.

The conjugation of LTA_4-methyl ester to LTC_4-methyl ester was studied based on the method described by Mannervik *et al.* [25] with the following modifications. Incubation mixtures in a total volume of 100 μL contained skin cytosol (25–200 μg protein) or affinity purified GST (5–20 μg protein) in 25 mM potassium phosphate buffer, pH 7.4, 0.5 mM EDTA and 5 mM GSH. The tubes containing reactants were kept at 37° for 1 min before and 1 min after the addition of GSH. The reaction was started by the addition of [^{3}H]LTA_4-methyl ester (final concentration 10 μM) and terminated after 10 min by the addition of 0.4 mL of ice-cold ethanol.

Quantitation of LTC_4-methyl ester formation. Quantitation of the reaction product was performed as described by Mannervik *et al.* [25]. The incubation mixture after the termination of the reaction was brought to 1.0 mL by the addition of water and extracted three times with an equal volume of ethyl acetate to remove LTA_4-methyl ester. LTA_4-GST activity was calculated from the radioactivity that remained in the aqueous phase after the ethyl acetate extractions. The amount of radioactivity in the aqueous phase was used as a measure of the formation of LTC_4-methyl ester, and was determined by counting the samples in a Packard Tri Carb 460 CD liquid scintillation counter equipped with automatic external standardization. Blanks without

the addition of protein were run simultaneously and data were corrected for the non-enzymatic reaction.

Identification of LTC_4-methyl ester formation by HPLC analysis and absorption spectroscopy. To establish the formation of LTC_4-methyl ester in the incubation mixture described above, a large scale incubation was carried out using affinity purified GST from rat skin cytosol. Portions of the total reaction mixture, and aqueous phase after extractions with ethyl acetate were analyzed on HPLC for the presence of LTA_4- and LTC_4-methyl esters. HPLC was performed on a C_{18} partisil column (250 × 4.6 mm) using methanol:water:acetic acid:phosphoric acid (75:25:0.08:0.02, by vol.) adjusted to pH 6.0 with NH_4OH (A) and 100% methanol (B) as the mobile phase. Solvent A was run for 20 min followed by solvent B at a flow rate of 1 mL/min, and the elution of the peaks was monitored by 280 nm absorbance. Fractions were collected every 0.5 min and 50-μL aliquots were used for determination of radioactivity. Additionally, the absorption spectrum of the aqueous phase, obtained after the ethyl acetate extractions of the incubation mixture, was also recorded. To further confirm the formation of LTC_4-methyl ester from LTA_4-methyl ester, the radioactive peak obtained from the HPLC of the aqueous phase after ethyl acetate extractions of the incubation mixture was collected, lyophilized and dissolved in 0.5 mL methanol containing 50% sodium hydroxide (9:1, v/v). The solution was kept at 4° for 5 hr, brought to pH 7.2 with dilute HCl, and subjected to HPLC under similar conditions as described above. Fractions were collected for radioactivity determination, and the radioactive peak (which also emerged as a single peak in 280 nm absorbance in HPLC profile) was collected, lyophilized and dissolved in 0.5 mL hexane to record its absorption spectrum.

RESULTS AND DISCUSSION

Incubation of LTA_4-methyl ester with rat, mouse or human skin cytosol in the presence of GSH resulted in the formation of LTC_4-methyl ester. Maximum enzyme activity was found to be associated with rat skin (20.2 ± 2.7 pmol/min/mg protein) followed by mouse skin (12.3 ± 0.9 pmol/min/mg protein). LTA_4-GST activity in human skin (5.9 ± 0.3 pmol/min/mg protein) was 2.1- to 3.4-fold lower than that in rodent skin. A lower rate of LTA_4-methyl ester conjugation with GSH in human skin may relate to the selectivity of the GST isozymes present in the human skin. Based on Western blot analysis and immunohistochemical studies, we have shown recently that in human and rodent skin GST Pi is the predominant isozyme expressed and that rodent skin contains a Mu class of isozyme whereas human skin contains an Alpha class of isozyme [16]. Thus, it is likely that the lower LTA_4-GST activity in human skin may be due to a low level or lack of the Mu class of isozyme which selectively conjugates LTA_4-methyl ester to LTC_4-methyl ester. Furthermore, utilizing immunohistochemical *in situ* localization, we have also shown that GST is predominantly localized in sebaceous glands and in the outer root sheath of the hair follicles, in the case of rodent as well as human skin [16]. The observations by Chang *et al.* [14, 15] have shown that the conjugation of LTA_4 to LTC_4 by purified GSTs from liver cytosol is maximal with the homodimer of the Yb subunit, viz. GST 4-4 and GST 3-3, followed by the heterodimer of the Yb subunit, viz. GST 3-4. In these studies it was shown that, in general, the isozymes containing the Yb subunits which belong to the Mu class of GST isozymes showed higher activity than the isozymes containing the Ya and/or Yc subunits (Alpha class of GSTs). Since the human skin contains undetectable levels of the Mu class of isozyme [16], the reduced rate of LTA_4-methyl ester conjugation to LTC_4-methyl ester appears to correlate with the expression of GST isozymes in human skin, while higher activity in rodent skin for LTA_4 conjugation to LTC_4 may be due to the presence of the Mu class of GST isozyme.

Using rat skin cytosol which exhibited maximum LTA_4-GST activity, we studied the subcellular distribution of the enzyme. Maximum enzyme activity occurred in the 100,000 *g* cytosolic fraction (20.9 ± 2.2 pmol/min/mg protein) followed by the 100,000 *g* microsomal fraction (13.3 ± 1.9 pmol/min/mg protein); however, the 800 *g* supernatant showed comparatively lower enzyme activity (5.2 ± 0.8 pmol/min/mg protein). A substantial rate of LTA_4-methyl ester conjugation to LTC_4-methyl ester by skin microsomes in addition to the cytosol is an interesting observation because the cytosolic

Table 1. LTA_4-GST activity in rat skin

System	LTA_4-GST activity* (pmol/min/mg protein)	% of Complete
Complete assay mixture†	20.2 ± 2.7	
Minus protein	2.2 ± 0.1	10.9
Minus GSH	0.8 ± 0.05	4.0
Boiled protein	2.8 ± 0.1	13.9

Enzyme activity was determined using [^{3}H]LTA_4-methyl ester as substrate, and LTC_4-methyl ester formation was measured as described in Materials and Methods.

* Each value is the mean ± SD of four assays conducted in triplicate.

† Complete assay mixture consisted of 100 μg cytosolic protein, 10 μM [^{3}H]LTA_4-methyl ester and 5 mM GSH.

Table 2. Purification of GST from rat skin cytosol

Substrate	Specific activity*		Fold purification
	Cytosol	Affinity purified GST	
CDNB	34.7 ± 2.9	1904.2 ± 15.9	55
Ethacrynic acid	4.4 ± 0.3	295.5 ± 12.2	67
[^{3}H]LTA$_4$-methyl ester	20.5 ± 1.9	248.2 ± 18.6	12

Enzyme activity in crude cytosol and affinity purified GST using CDNB, ethacrynic acid and [^{3}H]LTA$_4$-methyl ester as substrates was determined as described in Materials and Methods.

* Each value is the mean ± SD of four assays conducted in triplicate. When CDNB or ethacrynic acid was used as the substrate, specific activity is given in nmol/min/mg protein; when [^{3}H]LTA$_4$-methyl ester was used as the substrate, activity is given in pmol/min/mg protein.

and particulate GSTs in other tissues have been shown to exhibit quite different characteristics and substrate specificities [26]. It is likely that the microsomal enzyme activity observed in the present investigation represents LTC$_4$ synthase activity [27]. In this study, however, we did not pursue any further characterization of skin microsomal enzyme activity because the cytosolic enzyme was more active as far as LTA$_4$-methyl ester conjugation with GSH to LTC$_4$-methyl ester was concerned. The conjugation of LTA$_4$-methyl ester with GSH by skin cytosol was a GST-dependent process since the reaction exhibited a requirement for GSH and enzyme protein (Table 1). The use of boiled protein resulted in substantially lower conjugation of LTA$_4$-methyl ester (Table 1).

The molecular species of GST isozymes present in rat skin cytosol were characterized by utilizing affinity purified GST from rat skin cytosol. As shown in Table 2, by ammonium sulfate precipitation followed by affinity chromatography on GSH-agarose, skin GST was purified about 55-fold (with CDNB as a substrate), with a yield of 41%. The affinity purified GST exhibited 12- and 67-fold higher activity with LTA$_4$-methyl ester and ethacrynic acid, respectively, as compared to the crude preparation (Table 2). The SDS–PAGE analysis of the affinity purified GST showed a very prominent band at 22.5 kDa along with one major band at 26 kDa (Fig. 1). Few other minor contaminating bands in the high molecular weight region were seen in this preparation. In Western blot analysis of the affinity purified GST using polyclonal antibodies to GST Alpha, Mu and Pi, the 22.5 kDa protein reacted with the GST Pi antibody, while the 26 kDa protein reacted with GST Mu as well as GST Alpha (Fig. 2). These results indicate the presence of all three subclasses of GST isozymes in the affinity purified GST in which the predominant species was GST Pi, followed by GST Mu and GST Alpha.

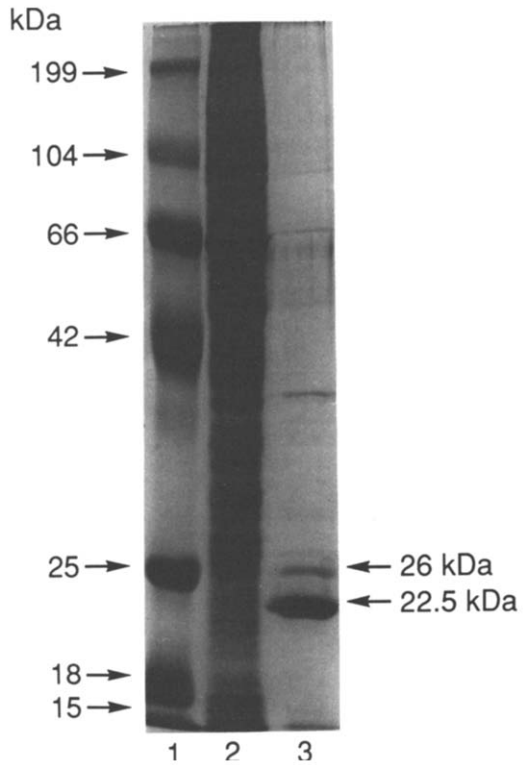

Fig. 1. SDS–PAGE analysis of affinity purified GST. Cytosol prepared from rat skin and GST purified from rat skin cytosol by GSH-agarose affinity chromatography were subjected to 12% SDS–PAGE as described in Materials and Methods. Key: lane 1, molecular weight markers; lane 2, 50 μg skin cytosol; and lane 3, 10 μg of partially purified GST.

The formation of LTC$_4$-methyl ester was identified by HPLC analysis of the incubation mixture using affinity purified GST as the source of the enzyme. As shown in Fig. 3 (top panel), the HPLC analysis of the total incubation mixture before ethyl acetate extraction exhibited two major peaks that eluted at about 8 and 32 min, as observed by 280 nm absorbance and radioactivity profiles. The peak that eluted at 32 min was identified as LTA$_4$-methyl ester using commercial standard (data not shown). However, when the incubation mixture was extracted with ethyl acetate before HPLC, the peak at 32 min totally disappeared (Fig. 3, bottom panel) which

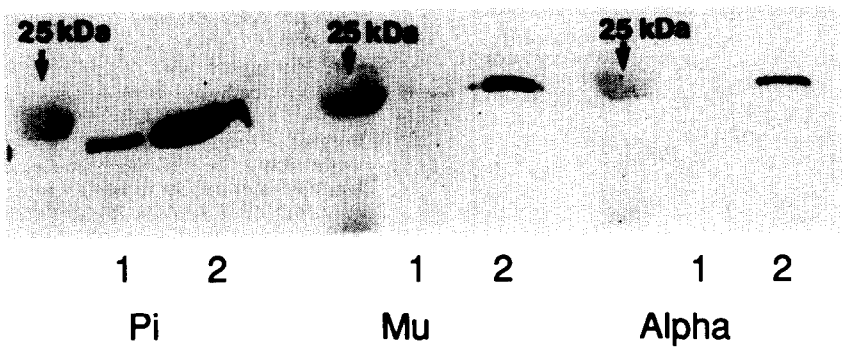

Fig. 2. Western blot analysis of affinity purified GST. Cytosol (70 μg protein) prepared from rat skin and affinity purified GST (20 μg protein) were subjected to 12% SDS–PAGE as described in Materials and Methods. The proteins were transferred onto nitrocellulose membrane according to Towbin *et al.* [23] and probed with the antibodies to GST Alpha, Mu and Pi as described earlier [16]. A 25 kDa molecular weight marker is shown on the extreme left. Key: lane 1, skin cytosol; and lane 2, affinity purified GST.

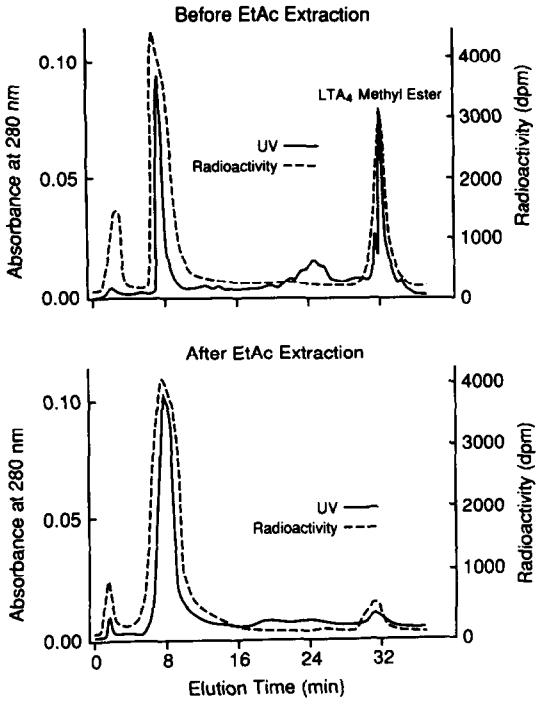

Fig. 3. Reverse-phase HPLC of the incubation mixture. Top and bottom panels show, respectively, the HPLC profile of incubation mixture before and after EtAc (ethyl acetate) extractions. For experimental details see Materials and Methods.

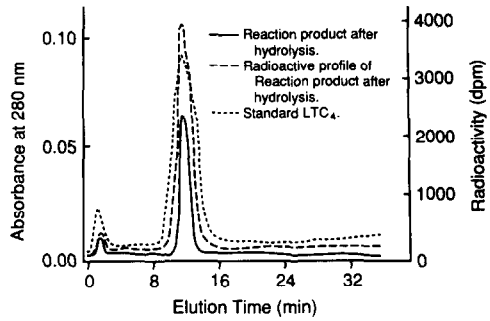

Fig. 4. Reverse-phase HPLC of the unknown peak eluted at 8 min in Fig. 3 after mild alkaline hydrolysis. For experimental details, see Materials and Methods.

further supported our assumption that this peak was LTA_4-methyl ester. The unknown peak with maximum radioactivity which eluted at 8 min in the HPLC of incubation mixture before and after ethyl acetate extractions (Fig. 3, top and bottom panels, respectively) was thought to be LTC_4-methyl ester. To further characterize this peak, it was digested under mild alkaline conditions and then subjected to HPLC analysis. As shown in Fig. 4, a new radioactive peak was eluted at about 12 min with the same retention time as that of standard LTC_4 (Fig. 4). This alkaline hydrolysis and identification of LTC_4 suggested that the unknown peak which eluted at 8 min (Fig. 3, top and bottom panels) was LTC_4-methyl ester.

The formation of LTC_4-methyl ester from LTA_4-methyl ester was further established by the absorption spectrum of the reaction product obtained from the large scale incubation mixture after ethyl acetate extractions. As shown in Fig. 5, the absorption spectrum for the reaction product showed a maximum absorbance at 280 nm with two shoulders at 270 and 292 nm, identical to that of standard LTC_4 [25]. Similarly, the alkaline hydrolysis product, shown in Fig. 4, also gave the same spectrum (Fig. 5), suggesting that the unknown compound formed from LTA_4-methyl ester was LTC_4-methyl ester.

In summary, in this study we have shown the conversion of LTA_4-methyl ester to LTC_4-methyl ester in rodent and human skin cytosol by a GST-

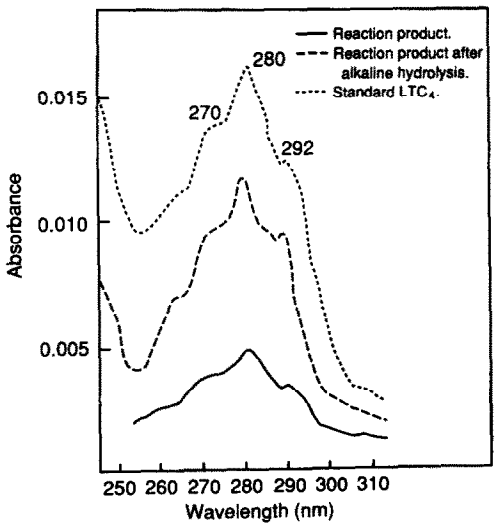

Fig. 5. Absorption spectra of standard LTC_4 and the reaction product before and after alkaline hydrolysis. For experimental details, see Materials and Methods.

dependent pathway. Although there are large kinetic differences between the physiological substrate LTA_4 and LTA_4-methyl ester, the results of the present study suggest that in skin disorders such as inflammation, and diseases such as psoriasis, where the levels of lipoxygenases are high [28], cutaneous GSTs may play an important role in metabolizing endogenous LTA_4, the product of 5-lipoxygenase-dependent metabolism of arachidonic acid [28]. Since, GST is localized predominantly in the sebaceous glands and the outer root sheath of the hair follicle [16], it can be assumed that GST-conjugated metabolism of LTA_4 to LTC_4 may be a transcellular metabolic process, and that the latter may be a potential source of substrate, specifically in the case of inflammatory cells.

Acknowledgements—This work was supported by USPHS Grants ES-1900, CA 51802, and P-30-AR-39750 and by research funds from the Department of Veterans Affairs.

REFERENCES

1. Samuelsson B, The leukotrienes: An introduction. In: *Advances in Prostaglandin, Thromboxane, and Leukotriene Research* (Eds. Samuelsson B and Paoletti R), Vol. 9, pp. 1–17. Raven Press, New York, 1982.
2. Samuelsson B, Leukotrienes, a new class of mediators of immediate hypersensitivity reactions and inflammation. In: *Advances in Prostaglandin, Thromboxane, and Leukotriene Research* (Eds. Samuelsson B and Paoletti R), Vol. 11, pp. 1–13. Raven Press, New York, 1983.
3. Haeggström J, Bergman T, Jörnvall H and Rådmark O, Guinea-pig liver leukotriene A_4 hydrolase: Purification, characterization and structural properties. *Eur J Biochem* **174**: 717–724, 1988.
4. Bigby TD, Lee DM, Meslier N and Gruenest DC, Leukotriene A_4 hydrolase activity in human airway epithelial cells. *Biochem Biophys Res Commun* **164**: 1–7, 1989.
5. Ford-Hutchinson AW, Arachidonic acid metabolism: Enzymatic pathways. In: *Eicosanoids and the Skin* (Ed. Ruzicka T), pp. 3–22. CRC Press, Boca Raton, 1990.
6. Borgeat P and Samuelsson B, Arachidonic acid metabolism in polymorphonuclear leukocytes: Unstable intermediate in formation of dihydroxy acids. *Proc Natl Acad Sci USA* **76**: 3213–3217, 1979.
7. Rouzer CA, Matsumoto T and Samuelsson B, Single protein from human leukocytes possesses 5-lipoxygenase and leukotriene A_4 synthase activity. *Proc Natl Acad Sci USA* **83**: 857–862, 1986.
8. Murphy RC, Hammarström S and Samuelsson B, Leukotriene C: A slow-reacting substance from murine mastocytoma cells. *Proc Natl Acad Sci USA* **76**: 4275–4279, 1979.
9. Piper PJ, Formation and actions of leukotrienes. *Physiol Rev* **64**: 744–755, 1984.
10. Dahinden CA, Clancy RM, Gross M, Chiller JM and Hugli TE, Leukotriene C_4 production by murine mast cells: Evidence of a role for extracellular leukotriene A_4. *Proc Natl Acad Sci USA* **82**: 6632–6636, 1985.
11. Ford-Hutchinson AW and Rackham A, Leukotrienes as mediators of skin inflammation. *Br J Dermatol* **109** (Suppl 25): 26–32, 1983.
12. Lewis RA and Austen KF, The biologically active leukotrienes. Biosynthesis, metabolism, receptors, functions and pharmacology. *J Clin Invest* **73**: 889–897, 1984.
13. Hammarström S, Örning L and Bernström K, Metabolism of leukotrienes. *Mol Cell Biochem* **69**: 7–16, 1985.
14. Chang M, Rao MR, Reddanna P, Li CH, Tu C-PD, Corey EJ and Reddy CC, I. Specificity of glutathione *S*-transferases in the conversion of leukotriene A_4 to leukotriene C_4. *Arch Biochem Biophys* **259**: 536–547, 1987.
15. Chang M, Hong Y, Burgess JR, Tu C-PD and Reddy CC, II. Isozyme specificity of rat liver glutathione *S*-transferases in the formation of $PGF_{2\alpha}$ and PGE_2 from PGH_2. *Arch Biochem Biophys* **259**: 548–557, 1987.
16. Raza H, Awasthi YC, Zaim MT, Eckert RL and Mukhtar H, Glutathione *S*-transferases in human and rodent skin: Multiple forms and species-specific expression. *J Invest Dermatol* **96**: 463–467, 1991.
17. Mukhtar H and Bickers DR, Drug metabolism in skin: Comparative activity of the mixed-function oxidases, epoxide hydrolase and glutathione-*S*-transferase in liver and skin of the neonatal rat. *Drug Metab Dispos* **9**: 311–314, 1981.
18. Bickers DR, Dutta-Choudhury T and Mukhtar H, Epidermis: A site of drug metabolism in neonatal rat skin. Studies on cytochrome P-450 content and mixed-function oxidase and epoxide hydrolase activity. *Mol Pharmacol* **21**: 239–247, 1982.
19. Simons PC and VanderJagt DL, Purification of glutathione *S*-transferase from human liver by glutathione-affinity chromatography. *Anal Biochem* **82**: 334–341, 1977.
20. Singh SV, Partridge CA and Awasthi YC, Rat lung glutathione *S*-transferases: Evidence for two distinct types of 22000-*M*, subunits. *Biochem J* **221**: 609–615, 1984.
21. Habig WH, Pabst MJ and Jakoby WB, Glutathione *S*-transferases: The first enzymatic step in mercapturic acid formation. *J Biol Chem* **249**: 7130–7139, 1974.
22. Laemmli UK, Cleavage of structural proteins during assembly of the head of bacteriophage T_4. *Nature* **227**: 680–685, 1970.
23. Towbin H, Staehlin J and Gordon J, Electrophoretic transfer of proteins from polyacrylamide gels to

nitrocellulose sheets. *Proc Natl Acad Sci USA* **76**: 4350–4354, 1979.
24. Bradford MM, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254, 1976.
25. Mannervik B, Jensson H, Ålin P, Örning L and Hammarström S, Transformation of leukotriene A_4 methyl ester to leukotriene C_4 monomethyl ester by cytosolic rat glutathione transferases. *FEBS Lett* **175**: 289–293, 1984.
26. Morgenstern R and DePierre JW, Microsomal glutathione transferase. Purification in unactivated form and further characterization of the activation process, substrate specificity and amino acid composition. *Eur J Biochem* **134**: 591–597, 1983.
27. Söderström M, Mannervik B and Hammarström S, Leukotriene C_4 synthase: Characterization in mouse mastocytoma cells. *Methods Enzymol* **187**: 306–312, 1990.
28. Ruzicka T (Ed.), *Eicosanoids and the Skin*. CRC Press, Boca Raton, 1990.