Formation of Murine Macrophage-derived 5-Oxo-7-glutathionyl-8,11,14-eicosatrienoic acid (FOG7) Is Catalyzed by Leukotriene C4 Synthase*

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John M. Hevko and Robert C. Murphy‡
From the Division of Cell Biology, Department of Pediatrics, National Jewish Medical and Research Center, Denver, Colorado 80206

5-Oxo-7-glutathionyl-8,11,14-eicosatrienoic acid (FOG7), a biologically active glutathione (GSH) adduct of the eicosanoid 5-oxo-eicosatrienoic acid (5-oxoETE), is the major metabolite formed within the murine peritoneal macrophage. The conjugation of GSH to electrophilic 5-oxoETE in vitro was found to be catalyzed by both soluble glutathione S-transferase and membrane-bound leukotriene C4 (LTC4) synthase. The cysteolic glutathione S-transferase-catalyzed products were not biologically active; however, the adduct formed from recombinant LTC4 synthase had identical mass spectrometric properties and biological activity to the macrophage-derived FOG7. The biosynthesis of FOG7 in the macrophage was inhibited by MK-886, a known inhibitor of LTC4 synthase, suggesting that this nuclear membrane-bound enzyme might be responsible for GSH conjugation to 5-oxoETE in the intact cell. Subcellular fractionation revealed that the microsomal fraction from the murine macrophage contained the enzyme responsible for FOG7 biosynthesis. Western blot analysis confirmed the presence of LTC4 synthase in the microsomal fraction that did not catalyze conjugation of GSH to 1-chloro-2,4-dinitrobenzene, indicating an absence of microsomal glutathione S-transferase activity. These results suggest that LTC4 synthase, thought to be specific for the conjugation of GSH to LTA4, can also recognize 5-oxoETE as an electrophilic substrate.

The enzymatic oxidation of arachidonic acid plays an important role in biology, leading to the production of a diverse family of biologically active eicosanoids, which typically carry information between cells, acting as intracellular chemical communicators of cellular activation. One pathway of arachidonic acid oxidation involves the addition of molecular oxygen to carbon-5 of arachidonic acid to afford 5-hydroperoxyeicosatetraenoic acid (1, 2), a reaction catalyzed by 5-lipoxygenase, but also a product of free radical oxidation of arachidonic acid. Leukotrienes are derived from the chemically reactive intermediate leukotriene A4 (LTA4), which is the product of a second 5-lipoxygenase-mediated reaction that utilizes 5-hydroperoxyeicosatetraenoic acid as substrate. LTA4 is transformed either into the neutrophil chemotactic leukotriene B4 (LTB4) (3) through the LTA4 hydrolyase-catalyzed addition of water to LTA4 (4) or by conjugation of the tripeptide glutathione (GSH) by LTC4 synthase to yield leukotriene C4 (LTC4) (5). LTC4 is rapidly metabolized through a series of peptidic cleavage reactions by ectoenzymes to the cysteinyl-glycine leukotriene D4 (LTD4) and the cysteine leukotriene E4 (LTE4) (6). These three cysteine leukotrienes were previously known as slow reacting substances of anaphylaxis (7) and are synthesized by several inflammatory cell types including the eosinophils, mast cells, basophils, macrophages, and platelets (8–10). As a family, cysteine leukotrienes possess potent biological activities causing contraction of various smooth muscles and have been implicated as mediators of acute hypersensitivity reactions including asthma (11).

Although considerable interest has focused attention on the leukotriene pathway of arachidonic acid metabolism within cells, it is now recognized that another family of equally potent eicosanoids is formed through the metabolism of 5-hydroperoxyeicosatetraenoic acid via the substrate 5-hydroxyeicosatetraenoic acid, which itself is metabolized into 5-oxoETE by a NADP+–dependent dehydrogenase in the neutrophil (12, 13). The discovery that 5-oxoETE is a potent chemotactic factor for the eosinophil has raised interest in this eicosanoid because of a suggested role for eosinophils in the pathogenesis of asthma (14, 15). Recently, a new biologically active cysteinyll 5-lipoxygenase product was structurally characterized as 5-oxo-7-glutathionyl-8,11,14-eicosatrienoic acid (FOG7), which was shown to be chemotactic for both the eosinophil and neutrophil (16). FOG7 and LTC4 are the only known biologically active GSH adducts of arachidonic acid and are, interestingly, isobaric, with a molecular mass of 625 daltons.

The conjugation of GSH to various endogenous and exogenous electrophilic and nephophiles is not uncommon in biological systems due to the presence of numerous glutathione S-transferases (GSTs). The GSTs make up a complex multigene family of proteins that play a central role in detoxifying electrophilic xenobiotics in nearly all species studied (17). The primary function of these proteins is to catalyze the nucleophilic conjugation of GSH to exogenous and endogenous electrophiles by effectively increasing the concentration of the thiolate anion near the substrate when held in the active site (18). GSTs are typically cytosolic enzymes; however, there are four known microsomal GSTs including microsomal GST-I (19), microsomal GST-II (20), microsomal GST-III (21), and microsomal GST-IV (22). The GSTs make up a complex multigene family of proteins that play a central role in detoxifying electrophilic xenobiotics in nearly all species studied (17). The primary function of these proteins is to catalyze the nucleophilic conjugation of GSH to exogenous and endogenous electrophiles by effectively increasing the concentration of the thiolate anion near the substrate when held in the active site (18). GSTs are typically cytosolic enzymes; however, there are four known microsomal GSTs including microsomal GST-I (19), microsomal GST-II (20), microsomal GST-III (21), and microsomal GST-IV (22).
crosomal GST-III (21), and LTA₄ synthase (5). LTA₂ synthase, the enzyme responsible for the biosynthesis of LTA₄, differs from conventional GSTs by its selectivity for LTA₄ and closely related analogs and failure to conjugate GSH to xenobiotics (22). LTA₄ synthase also exhibits differential susceptibility to inhibitors (24) and lacks immunoreactivity to antibodies for known GSTs (23). The purpose of the present investigation was to determine which of these enzymes was responsible for the 1.4-Michael addition of GSH to 5-oxoETE in vivo to afford biologically active FOG, in the murine peritoneal macrophage.

**EXPERIMENTAL PROCEDURES**

**Materials**—5-oxoETE, 5-[6,9,11,12,14,15-D]-[oxoETE (greater than 99 atom % D), LTA₄ methyl ester, and LTC₄ were purchased from the Cayman Chemical Co. (Ann Arbor, MI). Hanks’ balanced salt solution (HBSS) was purchased from Invitrogen. Indo-1/AM was obtained from Molecular Probes (Eugene, OR). All solvents were HPLC grade and obtained from Fisher.

**Preparation of Cytosolic and Microsomal Macrophage Fractions**—Preparation of peritoneal macrophages (see above) was carried out at 4 °C. Macrophages (80 x 10⁶/H₁₁₀₀₃ platelet-rich plasma was acidified by the addition of 1/10 volume of ACD (22) and leupeptin (5 x 10⁻⁵ M) and PMSF (10⁻⁵ M) were added to obtain fresh-elicited peritoneal macrophages (see above). Recombinant human LTC₄ synthase and LTC₄ synthase polyconal antibody were kind gifts from K. F. Austen and B. Lam (Harvard, Boston, MA). FOG was synthesized from peritoneal macrophages as previously described (16). The free acid of LTA₄ was synthesized by the hydrolysis of LTC₄ by H₂SO₄ as previously described (24).

**Collection of Elicited Peritoneal Macrophages**—Elicited macrophages were obtained by injecting 1 ml of thioglycolate 4% (10%) into the peritoneum of ICR mice. After 3 days, the mice were euthanized in a CO₂ atmosphere. The peritoneum was then lavaged once with 10 ml of Dulbecco's modified Eagle's medium, 10% fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin with 1% heparin. The peritoneal lavage fluid obtained was centrifuged at 600 x g for 8 min for the separation of cells from fluid.

**Isolation of Neutrophils**—Neutrophils were prepared from human peripheral blood in EDTA anticoagulant by dextran 60 sedimentation, contaminating erythrocytes were removed by centrifugation, and neutrophils were purified on Ficoll-Hypaque, yielding 96–97% neutrophils, 2–3% eosinophils, 0–1% mononuclear cells. All experiments were done within a 2-h in vitro age of the cells.

**Preparation of Human Platelets**—Peripheral blood was collected from healthy volunteers. The blood (20 ml) was treated with 2 ml of 72 mM EDTA (in saline). After two 15-min centrifugation steps at 1000 x g for 15 min at room temperature, the platelets were washed using the method of Patscheke (25) in citrate buffer, pH 6.5, containing 0.4% bovine serum albumin, 100 mM PGE₁, and MgCl₂. Platelets were resuspended in Hank’s buffer containing 1 mg/ml bovine serum albumin, pH 7.4, without Ca²⁺ and Mg²⁺ at 6 x 10⁷ platelets/ml.

**Preparation of Cytosolic and Microsomal Macrophage Fractions**—All steps below were carried out at 4 °C. Macrophages (80 x 10⁶ cells) in HBSS were centrifuged at 1,500 x g for 5 min. The pellet was washed with 10 mM HEPES buffer, pH 6.7, containing 137 mM NaCl, 2.6 mM KCl, 0.36 mM Na₂HPO₄, and 1 mM EDTA containing aprotinin (5 μg/ml) and leupeptin (5 μg/ml). The cell suspension was lysed by nitrogen cavitation (700 p.s.i., 20 min), and the lysis solution was centrifuged at 1,500 x g for 20 min to pellet cellular debris. The supernatant fraction was collected and centrifuged at 100,000 x g for 60 min. The pellet, containing microsomal enzymes (total protein 1.1 mg) was separated from the cytosolic enzymes in the supernatant (total protein 7.2 mg). The microsomal fraction was resuspended in HEPES buffer containing Triton X-100 (0.3%).

**GST-catalyzed Synthesis of GSH-5-oxoETE Adducts**—The addition of GSH (2 mM) to 5-oxoETE (10 μM) was carried out in HBSS with the presence of either human placental GST (5 units), rat liver GST (5 units), or recombinant human LTC₄ synthase (1.2 μg, partially purified from SF9 expression cells) for 15 min at 37 °C. GSH-5-oxoETE adducts were collected by centrifugation and separation using solid phase extraction. The above procedure was also carried out at pH 7.4 with D₅-5-oxoETE (10 μM) in the presence of human placental GST (5 units) to afford the D₅-5-oxoETE adduct internal standard (greater than 95% D₅).

**Effect of GSH-5-oxoETE Adduct Isolation and Purification**—The method supernatant, after solid phase extraction, was evaporated to dryness by vacuum rotary evaporation and redissolved in 60 μl of the internal HPLC mobile phase. Reverse phase HPLC was used to separate the GSH-5-oxoETE adducts by gradient elution with mobile phase A containing 8.3 mM acetic acid buffered at pH 5.2 with NH₄OH and mobile phase B composed of CH₃CN:methanol (65:35, v/v). GSH-5-oxoETE adducts were separated on a 150 x 2.0-mm Columbus 5-μm C₁₈ RP HPLC column (Phenomenex, Rancho Palos Verdes, CA), and fractions were collected at 1-min intervals from the column eluted at 200 μl/min with a linear gradient from 15% to 55% B in 5 min. Isolated fractions containing GSH-5-oxoETE adducts were analyzed by LC/MS/MS.

**Measurement of Cytosolic Calcium Levels**—Intracellular calcium was assessed by incubation of neutrophils (10⁶ cells/ml) loaded with the acetoxyethyl ester of Indo-1 (Indo-1/AM) as described previously (26). Before the addition of each test substance, CaCl₂ and MgCl₂ were added to the cell suspensions at 1 μM final concentration to 3 x 10⁶ neutrophils in a 4-ml cuvette. The Kₐ of 250 μM for the Indo-1/AM Ca²⁺ complex was used to calculate the intracellular calcium concentration, a Fₘₐₓ was determined by the addition of digitoxin at 0.1%, and Fₐₜ₉ was determined by the addition of 7.8 mM EDTA to this buffer.

**Actin Polymerization Determination**—Actin polymerization was assessed by flow cytometry as described previously (27). Briefly, neutrophils (0.9 ml of 1 x 10⁶ cells/ml) were incubated at 37 °C in HBSS in the presence of the test substance. After a 30-s incubation, cells were fixed, permeabilized, and stained in a single step by the addition of 0.1 ml of 37% phosphate-buffered formalin containing 1.65 x 10⁻⁶ M NBD-phal- licidin and 100 μg/ml of lysophosphatidylcholine. The stain mixture plus cells was incubated for 10 min at 37 °C. Cells were centrifuged at 400 x g for 5 min at room temperature and resuspended in HBSS (1 ml) before analysis.

**Enzyme Assays**—GST activity was measured using GSH and CDNB as substrates (28). The activity of the enzyme was determined in a 0.1 mM potassium phosphate buffer, pH 6.5, containing 1 mM GSH and 1 mM CDNB using an extinction coefficient of 9.6 μM⁻¹ cm⁻¹. The rate of product formation was monitored by measuring the change in absorbance at 340 nm. LTC₄ synthase activity was determined as previously described by measuring the formation of LTC₄ methyl ester by reversed phase HPLC after incubation of samples with LTA₄ methyl ester (10 μM) and GSH (2 mM) in HBSS (29). Protein concentrations were determined using the method described by Bradford (30). Gel electrophoresis and antibody recognition of LTC₄ synthase was carried out essentially as previously described (31) using a peptide antibody directed against GPPEFERYRAVQN in the sequence for LTC₄ synthase.

**Electrospray Mass Spectrometry (Negative Ions)**—Analysis of GSH-5-oxoETE and LTC₄ production was carried out using a Sciex API 3000 triple stage quadrupole mass spectrometer (PF Scientific, Canada). Multiple reaction monitoring of the specific transitions m/z 624 → 306, m/z 630 → 306, and m/z 624 → 272 were used to detect the elution of GSH-5-oxoETE adducts including FOG, the D₅-GSH-5-oxoETE adduct internal standard, and LTC₄ eluting from the HPLC.

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3 B. Lam, personal communication.
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RESULTS

FOG$_7$, in Vitro Synthesis—Cytosolic GSTs were examined as catalysts for conjugating GSH to 5-oxoETE via a 1,4-Michael addition reaction to afford product. For these studies, 5-oxoETE (10 $\mu$M) and GSH (2 mM) were incubated for 10 min at 37 °C in the presence of both commercial rat liver and human placental GSTs. The adducts were purified by solid phase extraction after the addition of D$_6$-GSH-5-oxoETE adduct internal standard and analyzed by LC/MS/MS (Fig. 1). Unique ion transitions were monitored for the GSH adducts and the internal standard such that production of adducts could be quantitated. In addition to investigating the catalytic properties of these cytosolic GSTs for 5-oxoETE conjugation, recombinant human LTC$_4$ synthase was also investigated for this activity. Multiple reaction monitoring revealed that all of the tested enzymes could catalyze the conjugation of GSH to 5-oxoETE; however, not all GSH addition products had the biological activity of FOG$_7$, as measured by F-actin polymerization using flow cytometry.

Recombinant LTC$_4$ synthase (Fig. 1A) produced a single product, observed by monitoring the transition m/z 624 → 306 by LC/MS/MS. This product eluted from the HPLC column slightly after that of the D$_6$-GSH-5-oxoETE adduct internal standard (m/z 630 → 306). This was the observed trend for FOG$_7$, biosynthesized by the peritoneal murine macrophage. The product catalyzed by LTC$_4$ synthase was observed to co-elute with macrophage-derived FOG$_7$ (data not shown).

The products catalyzed by both human placental (Fig. 1B) and rat liver cytosolic GSTs (Fig. 1C) produced similar results. Both the human and rat GSTs catalyzed the addition of GSH to 5-oxoETE to afford compounds that co-eluted with the authentic FOG$_7$, suggesting that these GSH adducts might be FOG$_7$. Furthermore, an additional minor adduct that eluted before that of D$_6$-GSH-5-oxoETE internal standard was observed using rat liver GST (Fig. 1C).

FOG$_7$ has been observed to profoundly activate chemotaxis and chemokinesis of both the human neutrophil and eosinophil by initiating polymerization of F-actin in these cells (16). The biological activity of the GSH-5-oxoETE adducts catalyzed by the cytosolic GSTs and the recombinant LTC$_4$ synthase was compared with FOG$_7$, for inducing F-actin polymerization in the human polymorphonuclear leukocyte. F-actin polymerization in neutrophils was induced by the LTC$_4$ synthase GSH-5-oxoETE adduct (Fig. 2A), but the human placental GST catalyzed product did not initiate the polymerization of F-actin in the human neutrophil (Fig. 2B), indicating that this latter GSH-5-
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The GSH-5-oxoETE adduct (50 nM) catalyzed by LTC$_4$ synthase, human LTC$_4$ synthase. To investigate whether or not LTC$_4$ catalytically active GSH-5-oxoETE adduct catalyzed by recombinant a retention time on RP HPLC identical to that of the biological adduct most likely was not chemotactic for these cells, contrary to that of macrophage-derived FOG$_7$. The two products catalyzed by the rat GST enzyme were separated by RP HPLC and analyzed for activity toward F-actin polymerization. Neither the major nor minor GSH-5-oxoETE adducts initiated polymerization of F-actin (Fig. 2, C and D). Flow cytometry revealed that the F-actin polymerization observed for FOG$_7$ (50 nM) afforded a shift in fluorescence almost identical to that of the GSH-5-oxoETE adduct (50 nM) catalyzed by LTC$_4$ synthase, again suggesting that this product might be FOG$_7$ and that LTC$_4$ synthase was perhaps the key enzyme in the biosynthesis of FOG$_7$.

Although 5-oxoETE had been observed to increase intracellular calcium in both the neutrophil and eosinophil as assessed using Indo-1/AM fluorescence, FOG$_7$ did not induce calcium mobilization in these cells (16). The GSH-5-oxoETE adducts produced by LTC$_4$ synthase and the cytosolic GSTs also showed no activity in regard to the elevation of intracellular calcium in the neutrophil (data not shown).

**Effect of MK-886 on GSH conjugation to 5-OxoETE**—The inhibitor MK-886 was tested as an antagonist for the conjugation of GSH to 5-oxoETE, catalyzed by recombinant LTC$_4$ synthase and both human and rat cytosolic GSTs. As observed for the LTC$_4$ synthase conjugation of GSH to LTA$_4$, MK-886 dose-dependently inhibited the addition of GSH to 5-oxoETE to afford the chemotactic adduct with an apparent IC$_{50}$ value of 7 $\mu$M (Fig. 3). The GSH-5-oxoETE adducts catalyzed by human and rat cytosolic GSTs were unaffected by MK-886 at concentrations up to 100 $\mu$M (Fig. 3).

When peritoneal murine macrophages were incubated for 15 min in the presence of 5-oxoETE (10 $\mu$M), a single GSH-5-oxoETE adduct resulted (previously referred to as FOG$_7$), with a retention time on RP HPLC identical to that of the biologically active GSH-5-oxoETE adduct catalyzed by recombinant human LTC$_4$ synthase. To investigate whether or not LTC$_4$ synthase, expressed in the macrophage, catalyzed the formation of FOG$_7$, the inhibitory effects of MK-886 were investigated in this cell. Elicited macrophages were incubated with 5-oxoETE (10 $\mu$M) at 37 $^\circ$C for 15 min in the presence of various concentrations of MK-886. As shown in a typical experiment (Fig. 4), MK-886 inhibited the formation of FOG$_7$ in the intact macrophage in a dose-related manner. The mean IC$_{50}$ value for the mouse enzyme was 7.1 $\pm$ 1 $\mu$M (mean $\pm$ S.E., n = 3).

For comparison, elicited macrophages incubated with LTA$_4$ (10 $\mu$M) at 37 $^\circ$C for 15 min afforded LTC$_4$, as identified by multiple reaction monitoring of the LTC$_4$-specific transitions $m/z$ 624 $\rightarrow$ 272. The production of LTC$_4$ in the elicited macrophage was inhibited by MK-886 in a dose-related manner (Fig. 4). The mean IC$_{50}$ value for this enzyme was 5.1 $\pm$ 1 $\mu$M (mean $\pm$ S.E., n = 3).

**Subcellular Localization of Enzymatic Activity toward FOG$_7$ Biosynthesis**—The location of FOG$_7$ biosynthesis in the murine macrophage was determined in crude cell lysate, cytosol, and membrane fractions. Each subcellular fraction was incubated with 5-oxoETE (10 $\mu$M) and GSH (2 mM) for 15 min at 37 $^\circ$C, and the extent of GSH conjugation to 5-oxoETE was analyzed by LC/MS/MS.

The total amount of protein recovered in both the cytosol and microsomal preparations was 83%. Both the cytosolic and microsomal fractions could catalyze the conjugation of GSH to 5-oxoETE, as evidenced by multiple reaction monitoring of aliquots from each of these samples. The microsomal fraction displayed a higher specific activity for the production of a GSH-5-oxoETE adduct than that observed from both the cell lysate and the isolated cytosolic fraction (Fig. 5). In addition to the higher specific activity, the GSH adduct from the microsomal fraction had a retention time identical to that of FOG$_7$, as witnessed by co-elution of these two compounds during RP HPLC (data not shown). Also this compound was capable of initiating actin polymerization (Fig. 6A), and the production of this compound was inhibited by MK-886 (Fig. 5). The GSH adduct catalyzed by macrophage cytosolic GSTs eluted before the D$_6$-GSH-5-oxoETE internal standard and as such, did not co-elute with authentic FOG$_7$ (data not shown) and did not initiate actin polymerization (Fig. 6B). In addition to this lack of activity, MK-886 had little or no effect on the production of this compound (Fig. 5), a result consistent with that for the cytosolic GSTs tested previously.

**Identification of LTC$_4$ Synthase in the Microsomal Fraction**—It appeared that the enzyme or enzymes responsible for the catalysis of GSH conjugation to 5-oxoETE to produce FOG$_7$ were membrane-bound proteins isolated in the microsomal...
fraction by fractional centrifugation. Whether LTC₄ synthase was the enzyme responsible for this process or whether additional microsomal GSTs were present in these cells and could catalyze this reaction was next examined.

Both the macrophage cell lysate, the cytosolic and the microsomal fractions (50 μg of protein), were assayed for LTC₄ synthase activity. The crude cell lysate and microsomal fraction catalyzed the conjugation of GSH to LTA₄-methyl ester to produce LTC₄-methyl ester. The cell lysate not only catalyzed the formation of the methyl ester, but also the formation of the free acid of LTC₄ by cytosolic esterases present in the macrophage. The cytosolic fraction did not catalyze formation of either LTC₄ or LTC₄ methyl ester.

Both the cytosolic and the microsomal fractions were assayed for LTC₄ synthase by Western blot analysis using peptide antibody raised against amino acids 65–78 in human LTC₄ synthase. This antibody had specificity toward the mouse LTC₄ synthase and revealed that this enzyme was present in the microsomal fraction and, as expected, was not detected in the cytosolic fraction (data not shown).

The apparent Kₘ and Vₘₐₓ for conjugation of glutathione to LTA₄ and 5-oxoETE was determined in microsomes isolated from the murine macrophage containing LTC₄ synthase. Various LTA₄ and 5-oxoETE concentrations were incubated with microsomal fractions (0.15 mg of protein/ml) after adding 2 mM GSH for 5 min before sample work-up essentially as previously described in studies of the LTC₄ synthase kinetics in platelets (10). Using hyperbolic regression analysis, the apparent Kₘ was found to be 1.3 ± 0.34 for LTA₄ and 1.6 ± 0.22 for 5-oxoETE, with Vₘₐₓ values for LTA₄ and 5-oxoETE of 89 ± 4.7 and 130 ± 3.7, respectively. These results represent the mean ± S.E. of three separate experiments.

To distinguish between microsomal GST-mediated catalysis and LTC₄ synthase catalysis in the membrane fraction, the conjugation of GSH to CDNB was examined. Recombinant LTC₄ synthase had no catalytic activity toward the conjugation of GSH with CDNB, as was the case for the microsomal fraction (Fig. 8). The cytosolic fraction, however, did possess the ability to conjugate GSH to CDNB, indicating that there are cytosolic GSTs present in the murine macrophage (Fig. 8).

**DISCUSSION**

The macrophage is an efficient cell in processing arachidonic acid with the formation of both cyclooxygenase (34) and lipoxygenase products (35, 36). Although the macrophage is also...
capable of producing the chemotactic eicosanoid 5-oxoETE, the synthesis of this eicosanoid is not significantly enhanced by cell stimuli in contrast to other 5-lipoxygenase products (37).

There is evidence to suggest that oxygenation of arachidonate at carbon-5 with formation of 5-hydroperoxyeicosatetraenoic acid, a precursor of 5-oxoETE, may be a preferred free radical pathway of arachidonate metabolism (38). The major metabolite of 5-oxoETE in the macrophage is the GSH 1,4-Michael addition product FOG7, which also has chemotactic properties (16). The macrophage expresses the unique microsomal protein, LTC4 synthase (39), long thought to be specific only for the human placental GST (1 unit), and recombinant human LTC4 synthase catalyzed the production of a single GSH-5-oxoETE adduct, which also has chemotactic properties (17). The macrophage expresses the unique microsomal protein, LTC4 synthase (39), long thought to be specific only for the human placental GST (1 unit), and recombinant human LTC4 synthase catalyzed the production of a single GSH-5-oxoETE adduct, which also has chemotactic properties (16). The macrophage expresses the unique microsomal protein, LTC4 synthase (39), long thought to be specific only for the human placental GST (1 unit), and recombinant human LTC4 synthase catalyzed the production of a single GSH-5-oxoETE adduct, which also has chemotactic properties (16).

Recombinant human LTC4 synthase as well as human and rat cytosolic GSTs all catalyzed the conjugation of GSH to the electrophilic 5-oxoETE, as identified by multiple reaction monitoring on a tandem quadrupole mass spectrometer. LTC4 synthase catalyzed the production of a single GSH-5-oxoETE adduct, which displayed identical chromatographic properties and biological activity to that of macrophage-produced FOG7. Both human and rat GSTs catalyzed the conjugation of GSH to 5-oxoETE to produce an adduct that was not separable from FOG7 under the RP HPLC conditions used; however, these adducts did not initiate F-actin polymerization, suggesting that these compounds were in fact not FOG7. Rat GST also produced an additional minor adduct that was separable from FOG7 under the RP HPLC conditions used; however, these adducts did not initiate F-actin polymerization, suggesting that these compounds were in fact not FOG7. Rat GST also produced an additional minor adduct that was separable from FOG7 under the RP HPLC conditions used; however, these adducts did not initiate F-actin polymerization, suggesting that these compounds were in fact not FOG7. Rat GST also produced an additional minor adduct that was separable from FOG7 under the RP HPLC conditions used; however, these adducts did not initiate F-actin polymerization, suggesting that these compounds were in fact not FOG7.

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CDNB + GSH (340 nm)

FIG. 8. GST activity for CDNB (1 mM) in subcellular fractions of the murine macrophage (5 µg of protein from each fraction). human placental GST (1 unit), and recombinant human LTC4 synthase incubated in 0.1 M potassium phosphate buffer, pH 6.5, containing GSH (1 mM), as measured by the change in absorbance at 340 nm over time using an extinction coefficient of 9.6 mM−1 cm−1.

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4 J. M. Hevko and R. C. Murphy, unpublished data.
macrophage or other cell types (e.g. platelet) without activation of 5-lipoxygenase and concomitant formation of LTA₄.

In summary, the biosynthesis of FOG₇ from 5-oxoETE and GSH in the elicited peritoneal macrophage was found to be catalyzed by a membrane-localized enzyme rather than cytosolic GSTs. Biochemical and pharmacological evidence suggests that nuclear membrane-bound LTC₄ synthase, long thought to be specific for the formation of LTC₄ from LTA₄, is responsible for this conjugation reaction.

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John M. Hevko and Robert C. Murphy

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