Leukotriene C₄ Is a Tight-binding Inhibitor of Microsomal Glutathione Transferase-1

EFFECTS OF LEUKOTRIENE PATHWAY MODIFIERS*

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Gerard Bannenberg§§, Sven-Erik Dahlén‡, Marjanka Luijerink¶, Gerd Lundqvist∥, and Ralf Morgenstern‡‡

From the Institute of Environmental Medicine, ÑDivision of Biochemical Toxicology and ÓDivision of Experimental Asthma and Allergy Research, Karolinska Institutet, Box 210, SE-17177 Stockholm, Sweden

Microsomal glutathione transferase-1 (MGST-1) is an abundant protein that catalyzes the conjugation of electrophilic compounds with glutathione, as well as the reduction of lipid hydroperoxides. Here we report that leukotriene C₄ is a potent inhibitor of MGST-1. Leukotriene C₄ was found to be a tight-binding inhibitor, with a Kᵢ of 5.4 nm for the unactivated enzyme, and 9.2 nm for the N-ethylmaleimide activated enzyme. This is the first tight-binding inhibitor characterized for this enzyme. Leukotriene C₄ was competitive with respect to glutathione and non-competitive toward the second substrate, CDNB. Analysis of stoichiometry supports binding of one molecule of inhibitor per homotrimer. Leukotrienes A₄, D₄, and E₄ were much weaker inhibitors of the purified enzyme (by at least 3 orders of magnitude). Leukotriene C₄ analogues, which have been developed as antagonists of leukotriene receptors, were found to display varying degrees of inhibition of MGST-1. In particular, the cysteinyl-leukotriene analogues SKF 104,353, ONO-1078, and BAYu9773 were strong inhibitors (IC₅₀ values: 0.13, 3.7, and 7.6 μM, respectively). In view of the partial structural similarity between MGST-1, leukotriene C₄ synthase, and 5-lipoxygenase activating protein (FLAP), it was of interest that leukotriene C₄ synthase inhibitors (which antagonize FLAP) also displayed significant inhibition (e.g. IC₅₀ for BAYx1005 was 58 μM). In contrast, selective 5-lipoxygenase inhibitors such as zileuton only marginally inhibited activity at high concentrations (500 μM). Our discovery that leukotriene C₄ and drugs developed based on its structure are potent inhibitors of MGST-1 raises the possibility that MGST-1 influences the cellular processing of leukotrienes. These findings may also have implications for the effects and side-effects of drugs developed to manipulate leukotrienes.

Glutathione transferases (see Refs. 1–4 for reviews) are a group of enzymes involved in the detoxification of numerous carcinogenic, mutagenic, toxic, and pharmacologically active compounds (5). A membrane-bound member of this family has been isolated (6) and named microsomal glutathione transferase-1 (MGST-1). This homotromeric enzyme has a distinct amino acid sequence and immunological properties that are different from its cytosolic counterparts (6, 7). Another discriminating property is its ability to be activated (up to 15-fold) by thiol reagents and proteinases (8, 9). An important function of the enzyme is thought to involve protection of intracellular membranes from oxidative modification as a result of oxidative stress (10), through the reduction of phospholipid and fatty acid hydroperoxides (11). A relationship between MGST-1 and the leukotriene pathway proteins leukotriene (LT) C₄-synthase and 5-lipoxygenase activating protein (FLAP) is indicated by similarities in size, hydropathy, and primary structure (12, 13). Furthermore, MGST-1 can physically interact with LTC₄-synthase (14, 15). Interestingly, additional members of this putative protein superfamily that display common functional properties have recently been described (13, 16).

Leukotriene C₄-synthase is a specialized membrane-bound glutathione transferase involved in the production of cysteinyl leukotrienes (LTC₄, LTD₄, and LTE₄). The cysteinyl leukotrienes are potent proinflammatory mediators that contribute to the pathophysiology of asthma. Hence, drugs which block formation or receptors for cysteinyl-leukotrienes are currently introduced as new therapy in asthma (17). LTC₄-synthase is homologous to FLAP (18).

Microsomal GST-1 has been identified as a high capacity “low affinity” binding protein for LTC₄ and was found to bind one molecule LTC₄ per trimer (19, 20). The purified protein did not display binding unless heat-inactivated microsomes had been added, implying the dependence on additional factors. The present study was undertaken to determine whether LTC₄ could in fact bind to MGST-1 under physiological conditions and whether such binding would influence the activity of the enzyme. Having established that this was indeed the case, we determined whether other leukotrienes and clinically developed inhibitors of leukotriene formation or receptors also were inhibitors of MGST-1. These studies indicate that interactions involving MGST-1 could have implications for leukotriene production and turn-over as well as for the effects or side-effects of drugs which are used in the treatment of asthma.

MATERIALS AND METHODS

Chemicals and Enzyme—Triton X-100, N-ethylmaleimide (NEM), glutathione (GSH), arachidonic acid, and fatty acid-free albumin were

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¶ To whom correspondence should be addressed. Tel.: 46-8-7287574; Fax: 46-8-334467; E-mail: ralf.morgenstern@imm.ki.SE.

1 The abbreviations used are: MGST-1, microsomal glutathione transferase-1; CDNB, 1-chloro-2,4-dinitrobenzene; CNBAM, 4-chloro-3-nitrobenzamide; FLAP, 5-lipoxygenase activating protein; LT, leukotriene; NEM, N-ethylmaleimide; NAC, N-acetyl-l-cysteine; PIPES, 1,4-piperazinediethanesulfonic acid.
from Sigma. CDNB and CNBAM were from Merck Co. (Darmstadt, Germany) and Alfred Bader Library of Rare Chemicals, Division of Aldrich Chemical Company (Milwaukee, WI), respectively. LTC₄, LTD₄, and LTE₄ were from Cascade Biochemicals, Reading, U.K. Zileuton (MK-679) was from Abbott Pharmaceuticals, Chicago, IL. U. 6,802,57 (piriprost) was from Pharmacia Upjohn, Kalamazoo, MI. BAYu9773 and BAYu9773 were from Bayer AG, Leverkusen, Germany. FPL 55712 was from Rhône Poulenc (previously Fisons Pharmaceuticals), Loughborough, U.K. ZD 230,487 and ICI 198,615 were from Zeneca Pharmaceuticals, Macclesfield, U.K. ONO-1078 (pranalolol) was from ONO Pharmaceuticals, Osaka, Japan; SKF 104,353 was from Smith-Kline Beecham, Philadelphia, PA; and MK591, MK886, MK-571, and MK-578 were from Merck Frosst, Montreal, Canada. LTA₄ was a kind gift from Dr. J. Haeggtorström, Department of Medical Biochemistry and Biophysics, Karolinska Institutet. All other chemicals were of the highest purity and were purchased from common commercial sources.

**Purification of MGST-1—** Microsomal GST-1 was purified from male Sprague-Dawley rat liver as described (21). The protein concentration was determined by the procedure of Peterson with bovine serum albumin as the standard (22). The purity of GST-1 was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 12.5% gels (23). A single band of molecular weight 17,000 was obtained after Coomassie Blue staining. Rat MGST-1 was used as a model for the human enzyme upon verification that LTC₄ is a strong inhibitor of the human enzyme.

**Measurement of MGST-1 Activity—** Microsomal GST-1 activity was measured according to Morgenstern et al. (24), by determination of the concentration of the product, GSH-S-conjugate with CDNB or CNBAM. Unless stated differently, all enzyme activity determinations were carried out in a standard buffer containing 100 mM potassium phosphate and 0.1% Triton X-100, pH 6.5, at 30 °C., with 5 mM GSH and 0.5 mM second substrate. The amount of enzyme used is indicated for each type of experiment. When the effect of calcium was determined, phosphate was exchanged for PIPES buffer to avoid precipitation.

**Enzyme Activation of Purified MGST-1 by LTC₄—** By NEM was performed as described earlier (21, 24). In short, the enzyme was incubated with 5 mM NEM on ice for up to 30 min; at maximal activation, the reaction was stopped by adding 5 mM GSH (adjusted to pH 7.0 by KOH).

**Proteoliposomes—** Unilamellar liposomes were prepared by sonication of 20 mg of phosphatidylcholine in 0.2 ml of 20% sodium cholate under a stream of nitrogen, until a clear translucent solution was obtained. The liposomes were diluted to 10 mg/ml with 10 mM potassium phosphate buffer; Ref. 21) was then added. The solution was dialyzed for 72 h against the dilution buffer containing 1 mM GSH and 0.05% sodium cholate, and for an additional 72 h omitting sodium cholate. The proteoliposome solution obtained contained 0.58 mg of protein/ml. Enzyme activity determinations were carried out in a standard buffer containing 100 mM potassium phosphate and 0.1% Triton X-100, pH 6.5, at 30 °C., with 5 mM GSH and 0.5 mM second substrate. The amount of enzyme used is indicated for each type of experiment.

When the effect of calcium was determined, phosphate was exchanged for PIPES buffer to avoid precipitation.

**Determination of the Mechanism, Stoichiometry, and Kᵢ of Inhibition of Purified MGST-1 by LTC₄—** The high affinity of LTC₄ for MGST-1 precluded analysis of the kinetics of inhibition by examining double-reciprocal plots. To determine the Kᵢ of inhibition and stoichiometry for LTC₄ binding to MGST-1, we employed the graphical method of Dixon (25). Inhibition by LTC₄ of unactivated and NEM-activated purified MGST-1 was determined at different concentrations of LTC₄. A graphical fit of empirical velocity measurements at various concentrations of inhibitor and in the absence of inhibitor (vₒ) was subsequently generated. Points on the curve were selected at vₒ/2, vₒ/3, vₒ/4, vₒ/5, and vₒ/6, and straight lines were generated through these points. The abscissa. The distance value between 0.5 mM GSH and 0.05% sodium cholate, and for an additional 72 h omitting sodium cholate. The proteoliposome solution obtained contained 0.58 mg of protein/ml. Enzyme activity determinations were carried out in a standard buffer containing 100 mM potassium phosphate and 0.1% Triton X-100, pH 6.5, at 30 °C., with 5 mM GSH and 0.5 mM second substrate. The amount of enzyme used is indicated for each type of experiment.

**RESULTS**

**Leukotriene C₄ Is an Inhibitor of Enzyme Activity—** Leukotriene C₄ strongly inhibited the enzymatic activity of MGST-1 incorporated into unilamellar phosphatidylcholine-containing liposomes (Fig. 1). The inhibition was concentration-dependent, and 50% inhibition was observed at approximately 50 nM LTC₄ at a protein concentration of 116 nM trimer. Clearly, the tight-binding previously observed (19) results in inhibition.

**Leukotriene C₄ Is an Inhibitor of Purified MGST-1 Solubilized in Triton X-100-containing Buffer—** To address the question of whether or not the presence of lipids is an absolute requirement for the binding/inhibition, we also investigated the effect of LTC₄ on the enzymatic activity of purified MGST-1 in Triton X-100-containing buffer. Also under these conditions, the inhibition was not observed as well as the NEM-activated MGST-1 was strongly inhibited by LTC₄ in a concentration-dependent fashion (Fig. 2, a and b).

**Mechanism of Inhibition of Enzymatic Activity of MGST-1 by LTC₄—** Leukotriene C₄ was found to be a tight-binding inhibitor, i.e. stoichiometric amounts of LTC₄ bound to the MGST-1 trimer, thereby inhibiting activity. The Kᵢ of inhibition with CDNB and GSH, or starting the reactions by addition of enzyme (26).

**To investigate the mode of inhibition of LTC₄, with respect to GSH and CDNB, competition experiments were performed. We employed a method for tight-binding inhibitor analysis as described by Henderson (27). The inhibitor concentration, divided by degree of inhibition (i(1 – vₒ/vᵢ)), was plotted against velocity without inhibitor divided by velocity with inhibitor (vₒ/vᵢ). The mechanism of inhibition was determined from the replots of the variation of the slope at different GSH (2, 5, 10, and 20 mM) or CDNB concentrations (50, 200, and 500 μM). These experiments were performed at 90 nM MGST-1 trimer. Because the plots deviated from linearity at low inhibition, this method was used to evaluate the type of inhibition in a qualitative manner, whereas the Dixon method (which does not rely on values at very low inhibition) was used for the quantitative estimations. Nevertheless, the Kᵢ value derived by the Henderson method (17 mM) agrees reasonably to that obtained with the Dixon method.
were generated from vo K of unactivated and N I used to determine Ki used as a substrate instead of GSH. The curve were selected at vo K of empirical velocity measurements (open squares curved line activated ( ) purified MGST-1. The purified enzyme was also inhibited by LTC4 if NAC was tested). Leukotriene A4 inhibited MGST-1 with an IC50 of 11 mM, not shown).

No difference in reaction velocities over time were seen whether the enzyme was preincubated with LTC4 before starting the reaction with CDNB and GSH, or starting the reactions by addition of enzyme (not shown), indicating that LTC4 does not inhibit MGST-1 by slow, tight-binding inhibition.

It has been reported that the binding of LTC4 to MGST-1 was dependent on divalent cations, such as calcium and magnesium (19). However, we found no such dependence nor any effect of either added calcium or magnesium on inhibition (0.2 μM–20 mM, not shown).

Other Leukotrienes and Arachidonic Acid Are Weaker Inhibitors of MGST-1 Activity—Leukotrienes D4 and E4 were much less potent inhibitors of MGST-1 than LTC4 (at least 3 orders of magnitude) with IC50 > 20 μM (higher concentrations were not tested). Leukotriene A4 inhibited MGST-1 with an IC50 of 11 μM. The inhibitory potency of LTA4 was very similar to that of arachidonic acid which displayed an IC50 of 9.7 μM (Table II).

Leukotriene Synthesis Pathway Inhibitors also Influence MGST-1 Activity—Among the inhibitors of leukotriene biosynthesis, three FLAP antagonists (MK-886, MK-591, and BAYx1005) and the compound piriprost (U 60,257) were found to cause significant inhibition of MGST-1 (Table III). The inhibitory potencies against MGST-1 were similar for all four inhibitors although piriprost and BAYx1005 appeared somewhat more potent. In contrast, zileuton and ZD 230,487 displayed minimal inhibitory activity (Table III).

With the exception of ICI 198,615, all tested antagonists of cysteinyl-leukotrienes inhibited MGST-1 (Table III). The leukotriene analogues ONO-1078, BAYu9773, and in particular SKF 104,353, were the strongest inhibitors of all tested compounds (Table III; Fig., 4). The profile of inhibitory activity did not relate to whether CDNB or CNBAM was the second substrate (Table III).

**DISCUSSION**

Published binding studies have indicated that MGST-1 contains a binding site for LTC4 (19). However, radiolabeled LTC4 was reported to bind to purified MGST-1 only if the binding incubations were supplemented with a Triton X-100 extract of dU937 cell membranes (that had been heat-inactivated to abol-

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**TABLE I**

| Activation Substrate | Ki (nM) | Stoichiometry |
|----------------------|--------|---------------|
| Unactivated GSH      | 5.4    | 1.25          |
| Activated GSH        | 9.2    | 0.83          |
| Unactivated NAC      | 4.4    | 0.74          |
| Activated NAC        | ND     | 0.83          |

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**FIG. 2.** Inhibition by LTC4 of unactivated (a) and N-ethyl maleimide activated (b) purified MGST-1. The curved line represents a manual fit of empirical velocity measurements (open squares) at various concentrations of inhibitor and in the absence of inhibitor (vi). Points on the curve were selected at vi/2, vi/3, vi/4, vi/5, and vi/6 and straight lines were generated from vi through these points to the abscissa. The distance values between the intersections of the straight lines (I0, I1, I2, and I3) with the abscissa were determined and averaged to obtain the Kave at the particular concentration of substrate. Kave was subsequently used to determine I1 and I0. The distance value between the origin and I0 on the abscissa estimates the concentration of binding sites present.

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**Fig. 3a** shows the results of inhibition of MGST-1 by LTC4. The purified enzyme was also inhibited by LTC4 if NAC was used as a substrate instead of GSH. The Ki for the unactivated enzyme was 4.4 nM (Table I) in this case. The Ki for the activated enzyme could not be determined, as only extrapolated data for the K_m (NAC) of activated MGST-1 exist. The stoichiometry of binding of LTC4 to the MGST-1 trimer was in all cases close to one, indicating that each trimer can bind one molecule of LTC4 (Table I).

Leukotriene C4 was a competitive inhibitor with respect to GSH. Slopes of the plot of inhibitor concentration divided by degree of inhibition (vi/vo) versus velocity without inhibitor divided by velocity with inhibitor (vo/vo) changed linearly with changes in GSH (Fig. 3a). Because inhibition was competitive, it must be reversible. As expected, the inhibition by LTC4 could be partially reversed also by addition of fatty acid-free albumin (maximally 30%, not shown). In contrast, LTC4 was a non-competitive inhibitor with respect to the second substrate CDNB (Fig. 3b).

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**Fig. 3** shows the results of inhibition of MGST-1 by LTC4. The purified enzyme was also inhibited by LTC4 if NAC was used as a substrate instead of GSH. The Ki for the unactivated enzyme was 4.4 nM (Table I) in this case. The Ki for the activated enzyme could not be determined, as only extrapolated data for the K_m (NAC) of activated MGST-1 exist. The stoichiometry of binding of LTC4 to the MGST-1 trimer was in all cases close to one, indicating that each trimer can bind one molecule of LTC4 (Table I).

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**Fig. 4** shows the results of inhibition of MGST-1 by LTC4. The purified enzyme was also inhibited by LTC4 if NAC was used as a substrate instead of GSH. The Ki for the unactivated enzyme was 4.4 nM (Table I) in this case. The Ki for the activated enzyme could not be determined, as only extrapolated data for the K_m (NAC) of activated MGST-1 exist. The stoichiometry of binding of LTC4 to the MGST-1 trimer was in all cases close to one, indicating that each trimer can bind one molecule of LTC4 (Table I).

Leukotriene C4 was a competitive inhibitor with respect to GSH. Slopes of the plot of inhibitor concentration divided by degree of inhibition (vi/vo) versus velocity without inhibitor divided by velocity with inhibitor (vo/vo) changed linearly with changes in GSH (Fig. 3a). Because inhibition was competitive, it must be reversible. As expected, the inhibition by LTC4 could be partially reversed also by addition of fatty acid-free albumin (maximally 30%, not shown). In contrast, LTC4 was a non-competitive inhibitor with respect to the second substrate CDNB (Fig. 3b).

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**Fig. 5** shows the results of inhibition of MGST-1 by LTC4. The purified enzyme was also inhibited by LTC4 if NAC was used as a substrate instead of GSH. The Ki for the unactivated enzyme was 4.4 nM (Table I) in this case. The Ki for the activated enzyme could not be determined, as only extrapolated data for the K_m (NAC) of activated MGST-1 exist. The stoichiometry of binding of LTC4 to the MGST-1 trimer was in all cases close to one, indicating that each trimer can bind one molecule of LTC4 (Table I).

Leukotriene C4 was a competitive inhibitor with respect to GSH. Slopes of the plot of inhibitor concentration divided by degree of inhibition (vi/vo) versus velocity without inhibitor divided by velocity with inhibitor (vo/vo) changed linearly with changes in GSH (Fig. 3a). Because inhibition was competitive, it must be reversible. As expected, the inhibition by LTC4 could be partially reversed also by addition of fatty acid-free albumin (maximally 30%, not shown). In contrast, LTC4 was a non-competitive inhibitor with respect to the second substrate CDNB (Fig. 3b).

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**Fig. 6** shows the results of inhibition of MGST-1 by LTC4. The purified enzyme was also inhibited by LTC4 if NAC was used as a substrate instead of GSH. The Ki for the unactivated enzyme was 4.4 nM (Table I) in this case. The Ki for the activated enzyme could not be determined, as only extrapolated data for the K_m (NAC) of activated MGST-1 exist. The stoichiometry of binding of LTC4 to the MGST-1 trimer was in all cases close to one, indicating that each trimer can bind one molecule of LTC4 (Table I).

Leukotriene C4 was a competitive inhibitor with respect to GSH. Slopes of the plot of inhibitor concentration divided by degree of inhibition (vi/vo) versus velocity without inhibitor divided by velocity with inhibitor (vo/vo) changed linearly with changes in GSH (Fig. 3a). Because inhibition was competitive, it must be reversible. As expected, the inhibition by LTC4 could be partially reversed also by addition of fatty acid-free albumin (maximally 30%, not shown). In contrast, LTC4 was a non-competitive inhibitor with respect to the second substrate CDNB (Fig. 3b).

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**Fig. 7** shows the results of inhibition of MGST-1 by LTC4. The purified enzyme was also inhibited by LTC4 if NAC was used as a substrate instead of GSH. The Ki for the unactivated enzyme was 4.4 nM (Table I) in this case. The Ki for the activated enzyme could not be determined, as only extrapolated data for the K_m (NAC) of activated MGST-1 exist. The stoichiometry of binding of LTC4 to the MGST-1 trimer was in all cases close to one, indicating that each trimer can bind one molecule of LTC4 (Table I).

Leukotriene C4 was a competitive inhibitor with respect to GSH. Slopes of the plot of inhibitor concentration divided by degree of inhibition (vi/vo) versus velocity without inhibitor divided by velocity with inhibitor (vo/vo) changed linearly with changes in GSH (Fig. 3a). Because inhibition was competitive, it must be reversible. As expected, the inhibition by LTC4 could be partially reversed also by addition of fatty acid-free albumin (maximally 30%, not shown). In contrast, LTC4 was a non-competitive inhibitor with respect to the second substrate CDNB (Fig. 3b).
Inhibition of MSGT-1 by Leukotriene C₄

Enzyme concentration was 89 nM trimer for the CDNB measurements and 520 nM trimer for the CNBAM measurements.

**TABLE III**
Leukotriene synthesis inhibitors and cysteinyl leukotriene receptor antagonists as inhibitors of MSGT-1 activity, assayed in vitro in 0.1 M potassium phosphate-buff fer containing 0.1% Triton X-100, pH 6.5, 30 °C

| Inhibitor | Inhibitory mechanism | CDNB | CNBAM |
|-----------|----------------------|------|-------|
| U (60,257) | 5-lipoxygenase | 140 ± 10 | 76 ± 4 |
| (pranlukast) | | | |
| ZD 230,487 | 5-lipoxygenase (active site) | >500b | >500c |
| (ileleuton) | (Fe-chelator) | | |
| BAYx1005 | FLAP | 58 ± 2 | 100 ± 10 |
| MK-591 | FLAP | 240 ± 40 | 240 ± 1 |
| MK-886 | FLAP | 190 ± 10 | 330 ± 20 |
| BAYu9773 | Receptor antagonist | 7.6 ± 0.7 | 41 ± 4 |
| FPL 55712 | Receptor antagonist | 18 ± 0.3 | 30 ± 2 |
| ICI 198,615 | Receptor antagonist | >250b | <250c |
| MK-571 | Receptor antagonist | 20 ± 2 | 33 ± 3 |
| MK-679 | Receptor antagonist | 110 ± 20 | 160 ± 10 |
| ONO-1078 | Receptor antagonist | 3.7 ± 0.2 | 3.0 ± 0.4 |
| SKF 8 | Receptor antagonist | 0.13 ± 0.03 | 0.82 ± 0.06 |

* * The IC₅₀ values were derived from plots of remaining activity versus inhibitor concentration as described under "Materials and Methods." Mean values ± S.E. of three independent experiments.
* * * Ileleuton, 12% inhibition at 500 μM; ZD 230,487, 16% at 125 μM; ICI 198,615, 25% at 250 μM.
* * * * Ileleuton, 11% inhibition at 500 μM; ZD 230,487, 14% at 125 μM; ICI 198,615, 29% at 250 μM.

**TABLE II**
Leukotrienes and arachidonic acid as inhibitors of MSGT-1 activity, assayed in vitro in 0.1 M potassium phosphate-buffer containing 0.1% Triton X-100, pH 6.5, 30 °C

| Compound | IC₅₀* (μM) |
|----------|------------|
| Arachidonic acid | 9.7 ± 0.9 |
| LTA₄ | 11 ± 0.4 |
| LTC₄ | 21 ± 1 |
| LTC₄ | 0.005 ± 4 μM |
| LTC₄ | >20μ |
| LTC₄ | >5μ |

* The IC₅₀ values were derived from plots of remaining activity versus inhibitor concentration as described under "Materials and Methods." IC₅₀ values ± S.E. of three independent experiments.

**Fig. 3.** Determination of the type of inhibition exerted by LTC₄. Shown is a replot of the slope of i/(1 - i/vₐ) versus vₐ/v₀ against concentration of GSH (a) or CDNB (b). Inset shows plot of i/(1 - i/vₐ) versus vₐ/v₀, at different concentrations of GSH and CDNB, respectively.

**TABLE I**
Inhibition of MSGT-1 by Leukotriene C₄

| Inhibitor | IC₅₀ (μM) |
|----------|----------|
| U (60,257) | 140 ± 10 |
| (pranlukast) | 76 ± 4 |
| ZD 230,487 | >500 |
| (active site) | >500 |
| A-64077 | >500 |
| (zileleuton) | (Fe-chelator) |
| BAYx1005 | 58 ± 2 |
| FLAP | 100 ± 10 |
| MK-591 | 240 ± 40 |
| FLAP | 240 ± 1 |
| MK-886 | 190 ± 10 |
| FLAP | 330 ± 20 |
| BAYu9773 | 7.6 ± 0.7 |
| Receptor antagonist | 41 ± 4 |
| FPL 55712 | 18 ± 0.3 |
| Receptor antagonist | 30 ± 2 |
| ICI 198,615 | >250 |
| Receptor antagonist | <250 |
| MK-571 | 20 ± 2 |
| Receptor antagonist | 33 ± 3 |
| MK-679 | 110 ± 20 |
| Receptor antagonist | 160 ± 10 |
| ONO-1078 | 3.7 ± 0.2 |
| Receptor antagonist | 3.0 ± 0.4 |
| SKF 8 | 0.13 ± 0.03 |
| Receptor antagonist | 0.82 ± 0.06 |

isi endogenous binding sites for LTC₄). A lipid component was therefore suggested to be required by purified MSGT-1 for binding LTC₄ (19). Our studies document that a lipid component is not required. Moreover, we discovered that LTC₄ was an extremely tight-binding inhibitor of MSGT-1. The Kᵢ of LTC₄ was 5.4 nM for the unactivated enzyme, and 9.2 nM for the NEM-activated enzyme. This agrees well with the previously estimated dissociation constant (19), although in our studies divalent cations are not required. In fact, the binding of LTC₄ was much stronger than that of GSH itself (Kᵢ = 18 μM) (28). The finding that activated and unactivated MSGT-1 display similar properties also agrees with previous observations on GSH and glutathione sulfonate binding (28). The activated enzyme did not exhibit an increased affinity for substrates or inhibitory ligands.

It was suggested that GSH at physiological concentrations should be able to displace LTC₄ from MSGT-1 (19). The present study contradicts these findings. The inhibitory capacity of low (near stoichiometric) levels of LTC₄ was well evident at 5 mM GSH (c.f. Fig. 1). Thus, physiological levels of GSH (1–10 mM; Ref. 29) will not effectively displace LTC₄ from MSGT-1. Rather, the amount of intracellular LTC₄ might determine the fraction of MSGT-1 available for catalysis. Furthermore, there is no absolute requirement for a lipid environment in order for LTC₄ to inhibit MSGT-1, as demonstrated by the strong inhibition of purified MSGT-1 dissolved in Triton X-100. Differences between the direct binding studies (19) and our kinetic data could perhaps be ascribed to some property of the binding assay used.

N-acetyl-L-cysteine is a useful analytical alternative to GSH because it is specifically utilized by MSGT-1 and not by cytosolic glutathione transferases (30). Here we demonstrate that LTC₄ inhibits MSGT-1 just as well when this alternative thiol donor is used to assay the enzyme. Therefore, NAC, in conjunction with LTC₄ inhibition should provide a helpful tool to determine the amount and activity of MSGT-1 in complex biological systems.

The binding of LTC₄ to MSGT-1 was reversible, as demonstrated by the reversibility of inhibition by fatty acid-free albumin. From the competition experiments, it appears likely that LTC₄ and GSH bind to the same binding site on MSGT-1 and that this site forms part of the active site of the enzyme.
Inhibition of MGST-1 by Leukotriene C₄

The high affinity binding of LTC₄ to MGST-1 stimulated us to explore whether or not compounds related to LTC₄, or drugs which inhibit the leukotriene pathway at different points in the cascade, interfered with MGST-1 activity. Many of the compounds indeed inhibited MGST-1.

The antagonists of the receptors for cysteinyl-leukotrienes were potent inhibitors of MGST-1. In particular, the compounds which may be classified as analogues of cysteinyl-leukotrienes (BAY u9773, SKF 104,353, and ONO-1078) were potent inhibitors of MGST-1. However, LTC₄ does not compete for the binding of CDNB, indicating that a fatty acid-binding site may be present in the MGST-1 trimer which is different from the hydrophobic substrate-binding site. Another likely explanation is the possibility that CDNB does not form a kinetically significant complex to MGST-1.

Previous studies have determined that there is one binding site for GSH per MGST-1 trimer (28). Tight-binding inhibitors can also be used as tools for the evaluation of stoichiometry. LTC₄ inhibition measured in the present study indicates that there is one LTC₄ binding site per trimer in agreement with direct binding studies with radiolabeled LTC₄ (19). The observed stoichiometry raises important issues regarding the enzymatic mechanism of MGST-1. Spatially equivalent overlapping mutually exclusive sites or non-overlapping sites can be envisioned in the homo-trimeric protein (31). If non-overlapping sites are present, conformational information upon binding of GSH or LTC₄ has to be transmitted, possibly resulting in alternating site catalysis. Structural studies presently performed are hoped to yield sufficient resolution to address these questions.

The high affinity binding of LTC₄ to MGST-1 stimulated us to explore whether or not compounds related to LTC₄, or drugs which inhibit the leukotriene pathway at different points in the cascade, interfered with MGST-1 activity. Many of the compounds indeed inhibited MGST-1.

The antagonists of the receptors for cysteinyl-leukotrienes were potent inhibitors of MGST-1. In particular, the compounds which may be classified as analogues of cysteinyl-leukotrienes (BAY u9773, SKF 104,353, and ONO-1078) were the most potent (Fig. 4). The potency of SKF 104,353 was striking, causing 50% inhibition in the submicromolar concentration range, which in fact suggests greater or equal affinity of this compound for MGST-1 in comparison with the CysLT₁ receptor (pA₂ values for SKF 104,353 against LTD₄ in guinea-pig and human airways being 8.6 and 8.0; Ref. 32). For BAY u9773 and ONO-1078, the activity against MGST-1 was also evident in the same concentrations as those required for antagonism of CysLT₁ receptors. However, there were also discrepancies between the compounds documented relative potency as pharmacological antagonists of the functional responses to cysteinyl-leukotrienes and the potency we observed as inhibitors of MGST-1. For example, the early prototype of CysLT₁-antagonists, FPL 55712, was as potent an inhibitor of MGST-1 as the considerably more potent leukotriene antagonist MK-571. Likewise, MK-679, which is the active enantiomer of the racemic compound MK-571, was 5-fold less active than MK-571. Moreover, ICI 198,615, which is the most potent antagonist of CysLT₁-receptors among the tested compounds (pA₂ values in different systems ranging between 10.1–9.3; Ref. 33), failed to cause significant inhibition of MGST-1.

Among the inhibitors of leukotriene formation, two relatively selective inhibitors of the 5-lipoxygenase (the iron-chelator zileuton and the active site inhibitor ZD 230,487) failed to inhibit MGST-1, whereas three different FLAP antagonists displayed significant potency as inhibitors (Fig. 4). The prostacyclin analogue piriprost inhibits leukotriene formation by mechanisms that have not been completely elucidated. Interestingly enough, interference with GST activity (34) has been discussed as one possible mechanism of its action, and piriprost was found to inhibit MGST-1 in this study.

The substrate for leukotriene formation, arachidonic acid, and the immediate precursor of LTC₄, LTA₄, both inhibited MGST-1, but their effects were only observed at relatively high concentrations. Successive reductions of the glutathionyl side chain in LTC₄ yields LTA₄ and LTE₄. Neither product was particularly potent as inhibitor of MGST-1, which is interesting because LTD₄ and LTE₄ are presumably formed extracellularly and not in the same compartments as MGST-1 and LTC₄.

Considered together, the new finding that not only LTC₄ but also certain antagonists and inhibitors of leukotrienes inhibited MGST-1 raises a number of questions relating to the function of MGST-1 and related intracellular proteins. There are, as discussed, observations suggesting physical proximity and interactions between FLAP, LTC₄ synthase and MGST-1. Our results, in addition, suggest previously unknown functional interactions between MGST-1 on the one hand and enzymes, substrates, products, and cofactors in the leukotriene pathway on the other hand. For example, binding of LTC₄ to MGST-1 may represent a means for transient storage of preformed LTC₄. Furthermore, binding of LTC₄ to MGST-1 may reduce product inhibition at the level of LTC₄ synthase, which may be desirable at times when there is a strong drive for leukotriene generation. Inhibition of enzymatic activity of MGST-1 by LTC₄ is also expected to interfere with the reduction of phospholipid and fatty acid hydroperoxides, which are important cofactors for the synthesis of leukotrienes and several other eicosanoids.

All in all, our findings generate the hypothesis that compounds which have some relation to FLAP or LTC₄ synthase also may interact with MGST-1. Tight binding of LTC₄ by another, cystolic, GST has also been observed (35). In this context, it should be noted that synthesis of LTC₄ will take place at the perinuclear membrane (36, 37), which means that intracellular proteins are exposed to LTC₄. In addition, transport mechanisms in the cell membrane which share affinity for LTC₄ and inhibitors of the leukotriene pathway have been described (38). Therefore, we suggest that our observations represent an indication that many different but related proteins must be considered in future equations and schemes attempting to comprehend how leukotrienes are formed and handled in the cell as well as released to the exterior with the aid of transport mechanisms. The observed interactions between LTC₄ and MGST-1, and in particular MGST-1 and several leukotriene antagonists, may in addition have bearing on the effects and side-effects of inhibitors of leukotrienes that currently are introduced as new treatment of asthma and other inflammatory diseases. For example, some drugs have caused...
liver function test abnormalities, and the effects we have observed in this study could well contribute to effects such as peroxisome proliferation that in fact has been documented for some of the drug candidates. The most potent inhibitors of MGST-1 we found could perhaps also be used to probe the role of the enzyme in cellular and organismal detoxification/oxidation reactions. For instance, the suggested role for MGST-1 in the protection against oxidative stress (10) and as the first step in the biotransformation of many nephrotoxic poly-halogenated hydrocarbons (39) can be further studied.

In conclusion, our findings have shown that LTC4 is a tight-binding inhibitor of MGST-1. This finding suggests an important role of MGST-1 in the intracellular management of LTC4. Future studies will therefore address the role of MGST-1 in the biosynthesis and intracellular transport of LTC4.

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REFERENCES

1. Armstrong, R. N. (1997) Chem. Res. Toxicol. 10, 2–18
2. Hayes, J. D., and Pulford, D. J. (1995) Crit. Rev. Biochem. Mol. Biol. 30, 445–690
3. Mannervik, B., and Widersten, M. (1995) in Advances in Drug Metabolism in Man (Pacifici, G. M., and Fracchia, G. N., eds) pp. 407–460, European Commission, Luxembourg
4. Mannervik, B., and Danielson, U. H. (1988) CRC Crit. Rev. Biochem. 23, 283–337
5. Chasseaud, L. F. (1979) Adv. Cancer Res. 29, 175–274
6. Morgenstern, R., Guthenberg, C., and DePierre, J. W. (1982) Eur. J. Biochem. 128, 243–248
7. Defong, J. L., Morgenstern, R., Jornvall, H., DePierre, J. W., and Tu, C.-P. D. (1988) J. Biol. Chem. 263, 8430–8436
8. Morgenstern, R., Lundqvist, G., Jornvall, H., and DePierre, J. W. (1989) Biochem. J. 260, 577–582
9. Morgenstern, R., DePierre, J. W., and Enzust, L. (1979) Biochem. Biophys. Res. Commun. 87, 657–663
10. Mestalou, E., Ekstrom, G., Adang, A. E. P., and Morgenstern, R. (1993) Biochem. Pharmacol. 45, 1645–1651
11. Mestalou, E., Piemont, P., Andersson, C., Vas, R., Van Bladeren, P. J., and Morgenstern, R. (1995) Arch. Biochem. Biophys. 320, 210–216
12. Weinander, R., Ekstrom, L., Raza, H., Lundqvist, G., Lindedal, B., Sun, T.-H., Hebert, H., Schmidt-Krey, I., and Morgenstern, R. (1999) in Glutathione S-transferases: Structure, Function and Clinical Implications (Vermeulen, N. P. E., Mulder, G. J., Nieuwenhuyse, H., Peters, W. H. M., and Van Bladeren, P. J., eds) pp. 49–56, Taylor & Francis Ltd., London
13. Jakobsen, P.-J., Mancini, J. A., and Ford-Hutchinson, A. W. (1996) J. Biol. Chem. 271, 22203–22210
14. Surapureddi, S., Morgenstern, R., Soderstrom, M., and Hammarstrom, S. (1996) Biochem. Biophys. Res. Commun. 229, 388–395
15. Soderstrom, M., Morgenstern, R., and Hammarstrom, S. (1995) Protein Expression Purif. 6, 352–356
16. Jakobsson, P.-J., Mancini, J. A., Riendeau, D., and Ford-Hutchinson, A. W. (1997) J. Biol. Chem. 272, 22934–22939
17. Holgate, S., and Dahlen, S. E. (eds) (1997) SRS-A to Leukotrienes: The Dawning of a New Treatment, Blackwell Science Ltd., Oxford
18. Ford-Hutchinson, A. W. (1994) Ann. N. Y. Acad. Sci. 744, 78–83
19. Metters, K. M., Sawyer, N., and Nicholson, D. W. (1994) J. Biol. Chem. 269, 12816–12823
20. Goffinet, A., and Nguyen, A. (1989) Eur. J. Pharmacol. 161, 99–101
21. Morgenstern, R., and DePierre, J. W. (1983) Eur. J. Biochem. 134, 591–597
22. Peterson, G. L. (1977) Anal. Biochem. 83, 346–356
23. Laemmli, U. K. (1970) Nature 227, 680–685
24. Morgenstern, R., Lundqvist, G., Hancock, V., and DePierre, J. W. (1988) J. Biol. Chem. 263, 6671–6675
25. Dixon, M. (1972) Biochem. J. 129, 197–202
26. Morrison, J. F. (1982) Trends Biochem. Sci. 7, 102–105
27. Henderson, P. J. F. (1972) Biochem. J. 127, 321–333
28. Sun, T.-H., and Morgenstern, R. (1997) Biochem. J. 326, 193–196
29. Cotgreave, I. A., and Moldeus, P. (1986) J. Biochem. Biophys. Methods 13, 231–249
30. Weinander, R., Andersson, C., and Morgenstern, R. (1994) J. Biol. Chem. 269, 71–76
31. Hebert, H., Schmidt-Krey, I., and Morgenstern, R. (1995) EMBO J. 14, 3864–3869
32. Hay, D. W., Mucitelli, R. M., Tucker, S. S., Vickers-Clark, L. M., Wilson, K. A., Glessner, M. G., Hall, R. F., and Torphy, T. J. (1987) J. Pharmacol. Exp. Ther. 243, 474–481
33. Snyder, D. W., Giles, R. E., Keith, R. A., Yee, Y. K., and Krell, R. D. (1987) J. Pharmacol. Exp. Ther. 243, 548–556
34. Bach, M. K., Brashler, J. R., White, G. J., and Galli, S. J. (1987) Biochem. Pharmacol. 36, 1461–1466
35. Sun, F. F., Chau, L. Y., Spur, B., Corey, E. J., Lewis, R. A., and Austen, K. F. (1986) J. Biol. Chem. 261, 8540–8546
36. Woods, J. W., Evans, J. F., Ethier, D., Scott, S., Vickers, P. J., Hearn, L., Heibein, J. A., Charleson, S., and Singer, I. (1993) J. Exp. Med. 178, 1935–1946
37. Peters-Golden, M., and McNish, R. W. (1993) Biochem. Biophys. Res. Commun. 196, 147–153
38. Leier, I., Jedditchsky, G., Buchholz, U., Cole, S. P. C., Deeley, R. G., and Keppler, D. (1994) J. Biol. Chem. 269, 27807–27810
39. Anders, M. W., and Dekant, W. (eds) (1994) Advances in Pharmacology, Vol. 27, Academic Press, San Diego
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Gerard Bannenberg, Sven-Erik Dahlén, Marjanka Luijerink, Gerd Lundqvist and Ralf Morgenstern

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