Crystal Structures of Leukotriene C<sub>4</sub> Synthase in Complex with Product Analogs

**IMPLICATIONS FOR THE ENZYME MECHANISM**

Received for publication, November 18, 2013, and in revised form, December 17, 2013. Published, JBC Papers in Press, December 23, 2013, DOI 10.1074/jbc.M113.534628

Damian Niegowski, Thea Kleinschmidt, Ulrika Olsson, Shabbir Ahmad, Agnes Rinaldo-Matthis, and Jesper Z. Haeggström

From the Division of Chemistry II, Department of Medical Biochemistry and Biophysics, Karolinska Institutet, S-107 77 Stockholm, Sweden

**Background:** Leukotriene (LT) C<sub>4</sub> synthase (LTC4S) is a membrane protein catalyzing the formation of pathogenic mediators of asthma.

**Results:** Crystal structures of complexes of WT and mutated LTC4S with three product analogs.

**Conclusion:** Evidence for the binding mode of LTA<sub>4</sub> and LTC<sub>4</sub> at the active site of LTC4S and a mechanistic model.

**Significance:** New insights into the molecular mechanism of LTC4S.

Leukotriene (LT) C<sub>4</sub> synthase (LTC4S) catalyzes the conjugation of the fatty acid LTA<sub>4</sub> with the tripeptide GSH to produce LTC<sub>4</sub>, the parent compound of the cysteinyl leukotrienes, important mediators of asthma. Here we mutated Trp-116 in human LTC4S, a residue proposed to play a key role in substrate binding, into an Ala or Phe. Biochemical and structural characterization of these mutants along with crystal structures of the wild type and mutated enzymes in complex with three product analogs, viz. 5-hexyl-, 4-phenyl-butyl-, and 2-hydroxy-4-phenyl-butyl-glutathione, provide new insights to binding of substrates and product, identify a new conformation of the GSH moiety at the active site, and suggest a route for product release, aided by Trp-116.

The leukotrienes are proinflammatory lipid mediators formed along the 5-lipoxygenase pathway of arachidonic acid metabolism (1). In this cascade, leukotriene (LT)<sup>4</sup> C<sub>4</sub> synthase (EC 4.4.1.20) catalyzes conjugation of the unstable epoxide intermediate LTA<sub>4</sub> ((5S)-trans-5,6-oxido-7,9-trans-11,14-cis-eicosatetraenoic acid) with GSH to form LTC<sub>4</sub> ((5S)-hydroxy-(6R)-S-glutathionyl-7,9-trans-11,14-cis-eicosatetraenoic acid), the parent compound of the cysteinyl leukotrienes C<sub>4</sub>, D<sub>4</sub>, and E<sub>4</sub>. These three compounds together comprise the classical slow reacting substance of anaphylaxis, an established mediator of human asthma (2, 3). Cysteinyl leukotrienes signal via two G-protein coupled receptors denoted CysLT1 and CysLT2 and antagonists of the former receptor are used in the clinical management of asthma (4–6). Unfortunately, ~40% of all patients do not respond to this treatment (7). Therefore, there is a need for more efficient therapeutic agents. Because recent data indicate that at least five different GPCR can transmit cysteinyl leukotriene signaling, the biosynthetic enzyme LTC4S has emerged as a promising target to eliminate mediator production and bioactions (8).

LTC4S is a trimeric integral membrane protein (Fig. 1) and a member of the MAPEG (membrane-associated proteins in gicosanoid and glutathione metabolism) family, which also includes three microsomal GSH S-transferases, 5-lipoxygenase activating protein, and microsomal prostaglandin E synthase 1 (9, 10).

Integral membrane proteins are essential in all living organisms for the shuttling of metabolites across lipid membranes as well as catalyzing enzymatic reactions. Knowledge of their structures, functions, and mechanisms is severely lacking compared with their soluble counterparts (Protein Data Bank) (12, 13). In particular, very little is known about the selectivity and specificity of proteins utilizing lipids as substrates within a lipid membrane. LTC4S and most MAPEG family members carry out their function in the interior of the lipid membrane and apart from having important interactions with constituents of the bilayer, which presumably are needed for stability and conformation, and they are also able to distinguish these lipid species from their in vivo substrates.

The crystal structure of human LTC4S has been solved and identified a unique horseshoe-shaped conformation of GSH bound at the active site by residues from two neighboring monomers (14, 15). It was postulated that the lipid substrate LTA<sub>4</sub> binds in a hydrophobic crevice between two monomers and that Trp-116 could act as a “molecular ruler” for binding of
LTA₄ with its ω-end buried under the aromatic side chain. Furthermore, mutational and spectroscopic studies have identified Arg-104 as the residue responsible for thiol activation (8, 16). In the present study, we explored the functional role of Trp-116 in human LTC₄S by mutational analysis and crystallography. As a surrogate for the labile endogenous leukotrienes (see Fig. 2), we synthesized three stable product analogs, viz. hexyl-, 4-phenylbutyl-, and 2-hydroxy-4-phenyl-butyl-glutathione, which thus carry a lipid tail to mimic the fatty acid backbone of LTA₄. We then determined the crystal structures of LTC₄S in complex with these compounds. Together, our results offer new insights to binding of LTA₄ at the active site and allow us to propose a model for product release.

**EXPERIMENTAL PROCEDURES**

**Materials**

Imidazole, Tris base, NaCl, KCl, Triton X-100, sodium deoxycholate, S-hexylglutathione-agarose, probenecid, GSH, and 2-mercaptoethanol were obtained from Sigma. Platinum Pfx DNA polymerase and deoxyribonucleotides were from Invitrogen. Dodecyl maltoside was obtained from Anatrace. LTA₄ methyl ester (BIOMOL) in tetrahydrofuran was saponified with 1M LiOH (6%, v/v) for 48 h at 4 °C. All other chemicals were obtained from common commercial sources.

**Site-directed Mutagenesis**

Site-directed mutagenesis was carried out according to the QuikChange protocol (Stratagene, La Jolla, CA) essentially as described (8). The primers for the W116A mutant were as follows: 5’-GCG GCG CGC GCC CTC GCG CTG GTG GCG-3’ (forward) and 5’-CGC CAC CAG CAG CGC GAG GGC GCG CGC CGC-3’ (reverse). For W116F, the primers were as follows: 5’-GCG GCG CGC GCC CTC GCG CTG GTG GTG GCG-3’ (forward) and 5’-CGC CAC CAG CAG CGC GAG GGC GCG CGC CGC-3’ (reverse). The integrity of the protein and the mutations were verified by DNA sequencing (SEQLAB, Göttingen, Germany).

**Protein Expression and Purification**

Wild type and mutated LTC₄S proteins were expressed in Pichia pastoris and purified with two steps of affinity chromatography essentially as described (15). For crystallization purposes, proteins were subjected to a buffer exchange on Superdex 200 16/60 (GE Healthcare) equilibrated with 25 mM Tris-HCl (pH 7.2), 0.03% (w/v) dodecyl maltoside, and 300 mM NaCl. Chromatographic fractions containing WT enzyme, W116A, and W116F LTC₄S were concentrated to 1–3 mg/ml by ultrafiltration. Protein concentrations were determined according to the method of Lowry (Sigma) with BSA as a standard. SDS-PAGE was performed on a PhastSystem (GE Healthcare) utilizing 10–15% gradient gels. Protein bands were visualized with Coomassie Brilliant Blue.

**Synthesis of Substrate Analogs**

**Analog I, ((S)-2-amino-5-((R)-1-(carboxymethylamino)-3-(hexylthio)-1-oxopropan-2-ylamino)-5-oxopentanoic acid)**

A suspension of reduced L-glutathione (3 g, 9.8 mmol) and 20 ml of 1M NaOH was prepared using a magnetic stirrer at room temperature. About 60 ml of 95% EtOH was added to the solution in small portions, until the “cloud point” was reached. L-iodo-hexane (1.5 ml, 10 mmol) was added, and the reaction was stirred at room temperature overnight. Next, the pH was adjusted to 3.5 with 67% hydriodic acid and the reaction mixture was cooled on an ice bath for a few hours, and a precipitation was formed, which was filtered off and washed with 10 ml of 95% EtOH and 100 ml cold water. The isolated precipitation was dried in vacuum to yield 2.6 g (6.6 mmol, 67%) of S-hexylglutathione as a white/light yellow powder.

**Analog II, ((S)-2-amino-5-((R)-1-(carboxymethylamino)-1-oxo-3-(4-phenylbutylthio)propan-2-ylamino)-5-oxopentanoic acid)**

4-Phenylbutan-1-ol (0.5 ml, 3.3 mmol), triphenylphosphine (1.15 g, 4.4 mmol), imidazole (0.31 g, 4.6 mmol), and iodine (1.14 g, 4.5 mmol) were dissolved in 100 ml Et₂O/MeCN 2:1 in a round bottom flask and then bubbled with Ar for a few minutes. The reaction was stirred at room temperature and followed by TLC toluene:EtOAc 6:1, showing complete conversion of starting material to product after 2.5 h. The reaction mixture was then washed with 3 × 50 ml H₂O, 2 × 50 ml saturated Na₂S₂O₃ and 2 × 50 ml saturated NaCl. The organic layer were dried with MgSO₄ (s), filtered, and evaporated to
give 0.55 g (2.1 mmol, 64%) 4-iodobutylbenzene as a white powder.

Reduced l-glutathione (0.37 g, 1.2 mmol) was dissolved in 2 ml 1 M NaOH. About 4.5 ml of 95% EtOH was added dropwise until the cloud point was reached. 0.32 g (1.2 mmol) of 4-iodobutylbenzene was added and the reaction was stirred at room temperature overnight. Hydroiodic acid was added dropwise until pH reached 3.5 and then cooled at 8 °C. The product mixture was kept at 8 °C to allow precipitation. The precipitated product was filtered and washed with water and EtOH and then dried under vacuum. The product was isolated as a white powder at a yield of 23% (0.13 g).

Analog III, ((2S)-2-amino-5-((2R)-1-(carboxymethylamino)-3-(2-hydroxy-4-phenylbutylthio)-1-oxopropan-2-ylamino)-5-oxo-oxopipanoic acid)—4-Phenyl-1-butene (1.3 ml, 8.7 mmol) was diluted in 10 ml dichloromethane at 0 °C. 3-Chloroperbenzoic acid (1.5 g, 25.6 mmol) was added in one portion, and the reaction mixture was stirred overnight without further cooling. The reaction was quenched and washed with 2 × 20 ml saturated NaHCO3 and further washed with by 2 × 20 ml water. The organic layer was dried with MgSO4 (s), filtered and, evaporated. The product was purified on a silica column giving 0.69 g (4.7 mmol, 54%) of (1-oxo-3-phenylethyl)oxirane as a white powder.

A suspension of reduced l-glutathione (2.13 g, 6.9 mmol) and 20 ml MeOH:triethylamine 4:1 was bubbled with Ar for 5 min. (S)-2-phenylethanolamine (0.68 g, 4.62 mmol) was added in one portion. The reaction mixture was then put in the oven at 37 °C for 24 h. The product mixture was cooled to room temperature and evaporated. The resulting white powder was dissolved in cold water and adjusted to pH 3 with 6 M HCl. The cloudy product suspension was then put in the fridge for 3 days to allow precipitation of the product. The product was filtered, washed with cold water, and dried under vacuum to give 0.87 g (1.92 mmol, 42%) of white crystals. All analogs were verified for the correct mass by electrospray ionization MS and fragmented by collision-induced dissociation.

**Enzyme Kinetics**

Enzyme activity toward GSH and LTA₄ for wild-type, W116F, and W116A LTC₄S was determined with aliquots of enzyme (0.1 μg for GSH and 0.05 μg for LTA₄) diluted to 100 μl with 25 mM Tris-HCl (pH 7.8) supplemented with 0.05% Triton X-100. When kinetic parameters for GSH and LTA₄ were determined, the buffer was supplemented with 36 μM of LTA₄ and 5 mM of GSH, respectively. The incubations were performed at room temperature and stopped after 15 s by the addition of 200 μl of methanol followed by 100 μl of water. Prostaglandin B2 (620 pmol) was added as an internal standard before reverse phase HPLC, which was performed on a Waters Nova-Pak C18 column.
Leukotriene C₄ Synthase in Complex with Product Analogs

Pak C18 column (3.9 x 150 mm) eluted with a mixture of acetonitrile/methanol/water/acetic acid (30:20:50:0.1, v/v) at an apparent pH 5.6 and at a flow rate of 0.8 ml/min (LKB 2150 pump). Qualitative analysis was performed by comparison with the retention time of synthetic LTC₄ using a Milton Roy SpectroMonitor 3100 detector. The amount of LTC₄ was quantified by calculating the ratio of the peak area compared with the internal standard prostaglandin B₂. The $k_{cat}$ and $K_m$ (Table 1) values were determined from the initial velocity of the LTC₄S-catalyzed reaction measured as a function of substrate (GSH or LTA₄) concentrations. The initial velocity data were fitted to the Michaelis-Menten equation using GraphPad Prism. The $k_{cat}$ value was calculated from the $V_{max}$ and enzyme concentration ([E]) according to the equation, $k_{cat} = V_{max}/[E]$ (see Table 1). For determination of IC₅₀ values of analogs I–III against WT or mutated LTC₄S, aliquots of LTC₄S (0.04–0.1 μg) were diluted to 100 μl with 25 mM Tris-HCl (pH 7.8), 0.05% Triton X-100, and 5 mM GSH and incubated with 30 μM LTA₄. Experimental data were fitted with the Michaelis-Menten equation using GraphPad Prism and RFFIT in SIMFIT to calculate the kinetic parameters $k_{cat}$, $K_m$, and $k_{cat}/K_m$ (SIMFIT software).

Crystallization

The crystals for the WT enzyme, W116F, and W116A LTC₄S were obtained using sitting drop vapor diffusion at room temperature at a concentration of 3.5 mg/ml. 1 μl of protein solution supplemented with 1 mM GSH were mixed with 1 μl of reservoir solution containing 1.8–2.2 M NH₄SO₄, 0.2 M NaCl, and 0.1 M sodium cacodylate, pH 6.1–6.8. Crystals were obtained after 1 day at room temperature and matured in size within 4 days. Soaks were conducted in the reservoir solution with the addition of 1 mM hexyl-GSH in time intervals ranging from 30 s to 24 h. The crystals were cryo-protected with the addition of 15% (v/v) glycerol in the presence of 1 mM analog I in the reservoir solution after which they were flash frozen in liquid nitrogen. For analogs II and III, a co-crystallization experiment was conducted. Protein was concentrated on an Amicon Ultra-10 filter (Millipore) and then 1 mM of analog was added and incubated for 20 min. After a quick high speed spin the protein was subjected to crystallization trials.

Data Processing and Refinement

Data were collected at the Helmholtz-Zentrum Berlin, BESSY beamline 14.1 (W116A mutant), the European Synchrotron Facility beamline ID14.4 (W116F mutant and LTC₄S in complex with analog II and III), and the European Synchrotron Facility beamline 23.2 (LTC₄S-analog 1 complex). The data were processed using XDS and scaled with SCALA (18, 19). Data cut-off was chosen with the new assessment criteria based on $1/2$ CC (20). It is worth mentioning that even when employing the classical criteria for data cut-off of $I/σ$ and $R_{merge}$ the resolution was only marginally worse, and little or no changes were observed in the electron density maps. The structure was solved using molecular replacement with PHASER using a modified PDB code 2UUI with waters and lipids removed (21). Refinement and simulated annealing was carried out with REFMAC and the PHENIX suite (22, 23). To remove possible model bias, 25 macrocycles of simulated annealing were carried out prior to model building and ligand introduction with a starting temperature of 5000 K. Model building was done using Coot (24). The analogs were identified from the original maps and also confirmed using $Fo-Fc$ maps calculated with FFT from PDB codes 2UUI, 2UUH, and 3PCV (data not shown). All figures were produced using PyMOL software (25). X-ray statistics are presented in Table 2.

RESULTS AND DISCUSSION

LTCA₄S is a highly specialized GSH S-transferase that converts LTA₄ into the spasmogenic LTC₄, an established asthma mediator. This enzyme is one of very few human integral membrane proteins whose structure has been solved at high resolution (15). Here, we combined mutational analysis with organic synthesis and crystallography to gain insights to the molecular mechanisms of LTC₄S catalysis.

Binding of Analog I–III Mimic of LTA₄ and LTC₄ Binding—

The lipid substrate LTA₄ is highly unstable ($t\frac{1}{2} \approx 10$ s) and cannot be used for co-crystallizations. To get a better picture of
how the substrate and product bind at the active site and probe the role of Trp-116, we synthesized three mimics of an LTA₄-GSH adduct, termed analogs I–III (Fig. 2). We solved the structures of wt LTC4S in complex with analogs I, II, and III to 2.75, 3.2, and 2.9 Å. Furthermore, the mutants W116A and W116F LTC4S structures were solved at 2.7 and 2.4 Å, respectively with analog I.

The $2F_o - F_c$ maps with analogs I–III after refinement are shown in Figs. 3 and 4. The analogs were identified as strong positive residual density in the $2F_o - F_c$ maps after simulated annealing and initial refinement but before ligand introduction (data not shown). The phenyl ring in analogs II and III were added to increase the visibility of the acyl chain that was to some extent disordered in the analog I complex structure, as well as to stabilize its interactions between the protein and the acyl/phenyl moiety via $\pi$-stacking to Trp-116. The 2-hydroxy group in analog III was introduced to mimic the LTA₄ epoxide opening in an attempt to shed light on the amino acid that would act as an acid during the catalytic reaction. In previous studies, Arg-31 was suggested to play that role (16). Analog III seems to result in tighter binding, as judged by the electron density and ability to inhibit enzyme activity; IC₅₀ values for analog III were 0.4–0.8 mM as compared with 2.3–4.3 mM for analog I. A possible explanation for this might be the ability to form a weak hydrogen bond between the 2-hydroxy group of analog III to the carbonyl oxygen of Ala-20 in the adjacent monomer at $\sim3.6$ Å distance, an interaction that did not, however, provide new information about epoxide opening in LTC4S. The binding conformation, however, remains the same for all three analogs. Analog II showed a higher than expected IC₅₀ value ($\sim10$ mM). The hydrophobic tails of all three analogs sit in the hydrophobic crevice created between two monomers and reach into the hydrophobic pocket under Trp-116 in which the $\omega$-end of LTA₄ is thought to bind and where a DDM molecule is bound in PDB code 2UUH. It has previously been suggested
that this DDM molecule might be a good mimic for binding of \textit{LTA}_4. In light of the binding conformation of the analogs in the complex structures, we believe that the position of the aliphatic side chain of DDM represents \textit{LTA}_4 binding at an early step of catalysis where the thiolate anion attacks C6 of the epoxide ring, whereas the three analogs used here mimic the product as it is leaving the active site (see below).

\textit{Trp-116 Is Not Essential for Catalysis}—We exchanged Trp-116 in LTC4S for an Ala or Phe residue and analyzed the effects on catalysis and kinetic parameters (Table 1). Considering the inherent variability that comes with enzyme assays with highly unstable lipid substrates, no major kinetic alterations were observed, thus demonstrating that Trp-116 is not essential for catalysis. The 3-fold increased \textit{LTA}_4 turnover of the W116F mutant may reflect a Phe configuration, which leaves the active site open to the lipid bilayer, thus facilitating product release (see below). The increased \textit{K}_m values of W116A/W116F for GSH are probably indirect effects of changes in \textit{LTA}_4 binding. The higher activity (two to four times) observed when LTA-4 is varied as compared with when GSH is varied, is most likely due to a gradual loss of active enzyme as the concentration of GSH is reduced.

\textit{Trp-116 Helps Position the \textit{ω}-End of \textit{LTA}_4}—To further probe the role of Trp-116 for the positioning of \textit{LTA}_4 at the active site, we solved the structures of W116A and W116F LTC4S in complex with analog I, at 2.7 and 2.4 Å resolution, respectively. Comparing the omit map densities between the WT structure and the W116A mutant, it is clear that the acyl chain of analog I is less ordered (data not shown), which coincides well with previous proposals that the function of Trp-116 is to anchor the tail of \textit{LTA}_4. Hence, Trp-116 appears to aid in the positioning of the \textit{ω}-end of \textit{LTA}_4, but this substrate–enzyme interaction seems to have very little, if any, impact on substrate turnover (Table 1).

Of special note, in the structure of the mutant W116F, the phenyl ring has adopted a tilted conformation due to rotation at the \(\alpha\)-\(\beta\) and \(\beta\)-\(\gamma\) carbon bonds (Fig. 3C). This rotamer opens the protein cavity toward the lipid bilayer. In addition, we have observed a similar twist of the natural Trp-116 in structures of the analogs, the terminal carboxylates on the \textit{LTC}_4S moiety of analog I (C) and GSH (D) are shown. Molecules are viewed from different perspectives, and rotations are indicated relative (A). Yellow dashed lines show distances. An asterisk indicates a residue from a neighboring monomer.

\textit{The GSH Moieties of Analog I–III Bind in a Tilted Conformation}—Examination of the active sites in the five analog complex structures reveals a shift in the binding configuration of the GSH part that is similar for all three analogs (Figs. 3 and 4). To detail binding interactions and analog positions, we used the structures of LTC4S and the mutant W116F in complex with analog I because they have the best interpretable electron densities. Binding of analog I at the active site of LTC4S is depicted in Fig. 5A. The coordinating residues are basically the same in our analog complex structures as compared with PDB code 2UUH with bound GSH. For both GSH and the GSH moiety of the analogs, the terminal carboxylates on the \(\gamma\)-glutamyl residue are coordinated by Arg30 and Gln53 from the adjacent monomer (Fig. 5A).

\textbf{FIGURE 5. Comparison between binding interactions of analog I (PDB code 4JCZ) and GSH (PDB code 2UUH) at the active site of LTC4S.} The overall coordinating network for analog I at the active site (A). Enzyme rearrangements at the active site with side chains in the complex structure with GSH depicted in 

\textit{FEBRUARY 21, 2014}
WT structure they overlap with the DDM acyl chain as well as the phenyl rings of analogs II and III. In the W116A and W116F mutants, the three terminal carbons shift by ~3.5 Å upwards toward the position of the Trp-116. This is most likely due to the larger space available in the absence of the tryptophan residue. In the W116F mutant, the phenylalanine is now pointing outwards to the solution/lipid bilayer and no longer functions as a “lid” for the hydrophobic pocket as seen with the Trp-116 in the WT structure.

**Potential Mechanism for Product Release**—Enzymes involved in fatty acid metabolism face a chemical problem in that lipids are poorly soluble in the cytoplasm or other aqueous compartments, which require special arrangements to allow substrate entry and product egress. Certain enzymes, for instance phospholipases and cyclooxygenases, cope with these problems through anchoring structures by which they get in close physical contact with the membrane compartment to make possible transport of lipids across the protein-membrane interphase (26, 27). For LTC4S, these mechanistic issues are even more complex. The entire protein is embedded in the lipid bilayer, and the enzyme handles two substrates with opposite chemical properties. GSH is a highly soluble tripeptide and...
must reach the GSH binding site located in between two monomers under the lipid bilayer (Fig. 1), whereas LTA₄ is a hydrophobic fatty acid that also carries a highly reactive aliphic epoxide, the binding site of which appears to be a superficial hydrophobic cleft below the membrane-protein interphase. Once the conjugation of GSH with LTA₄ has occurred, the resulting product (LTC₄) possesses both hydrophobic (GSH moiety) and hydrophobic (LTA₄ moiety) properties (Fig. 2). Our data offer clues to the manner in which LTC₄S manages these mechanistic problems.

The tilted configuration of the GSH moiety of the analogs is intriguing and is not observed when comparing structures of other GSTs having either GSH (PDB codes 1PKW and 3G71), hexyl-GSH (PDB codes 2R3X, 3F63, 3F6D, 17GS, and 3PRN), glutathione sulfonic acid (PDB code 1GLP), or ω-γ-glutamyl-3-sulfino-1-allylglycine (PDB code 1YZX) bound (Fig. 6). In these structures, the GSH part seems to overlap regardless of which other GSH analog is used. Although the above proteins are all soluble, the overall reactions they perform are the same, i.e. conjugation of the hydrophilic GSH to a wide array of hydrophobic compounds (11, 28, 29). Recently, a high resolution structure of another MAPEG family member, microsomal prostaglandin E synthase 1 in complex with the analog ω-γ-glutamyl-5-(2-biphenyl-4-yl-2-oxoethyl)-l-cysteinylglycine, was solved (17). In this structure, the GSH part of the analog overlaps with the GSH bound in the native structure (Fig. 6). This analog displays a very high degree of similarity with analogs II and III. Thus, it is tempting to speculate that the tilted conformation of the GSH moiety observed in our complex structures is a special feature of LTC₄S and reflects specific facets of the catalytic mechanism.

If one tries to integrate the results of our mutational analysis and structural information, one may speculate that after initial binding and activation of GSH, LTA₄ slides into the hydrophobic crevice and reacts with the GSH thiolate at a point indicated by DDM binding (Fig. 7). The product then continues to slide toward Trp-116, as indicated by the tilted conformation of analogs II and III. Further studies are required to detail the discrete steps in the enzymatic conjugation of LTA₄ with GSH, catalyzed by LTC₄S.

Summary—To detail the role of Trp-116 for substrate binding and catalysis, we mutated this residue into an Ala or Phe residue and characterized the mutated enzymes. Together with crystal structures of wt and mutated LTC₄S in complex with synthesized product analogs these data provide new insights to binding of substrates and product, identify a new conformation of the GSH moiety at the active site, and suggest a route for product release into the lipid bilayer, aided by Trp-116.

Acknowledgments—The authors acknowledge the European Synchrotron Facility for providing access to x-ray sources and especially local contacts at beamline 23.2 and ID14.4 for assistance. We thank Helmholtz-Zentrum Berlin for the allocation of synchrotron radiation and assistance at beamtime 14.1 as well as BiostuctX-783. We also thank the Protein Structure Facility at the Karolinska Institute for providing excellent facilities for protein crystallization.

REFERENCES

1. Peters-Golden, M., and Henderson, W. R. (2007) Mechanisms of disease: Leukotriene Pathways: Biochemistry, Biology, and Roles in Disease. Rev. Chem. 111, 5866–5898
2. Samuelsson, B. (2012) Role of Basic Science in the Development of New Medicines: Examples from the Eicosanoid Field. J. Biol. Chem. 287, 10070–10080
3. Drazen, J. M. (1999) Treatment of asthma with drugs modifying the leukotriene pathway. N. Engl. J. Med. 340, 197–206
4. Heise, C. E., O’Dowd, B. F., Figueroa, D. I., Sawyer, N., Nguyen, T., Im, D. S., Stocco, R., Bellefeuille, J. N., Abramovitz, M., Cheng, R., Williams, D. L., Jr., Zeng, Z., Liu, Q., Ma, L., Clements, M. K., Coulombe, N., Liu, Y., Austin, C. P., George, S. R., O’Neill, G. P., Metters, K. M., Lynch, K. R., and Evans, J. F. (2000) Characterization of the human cysteinyl leukotriene 2 receptor. J. Biol. Chem. 275, 30531–30536
5. Lynch, K. R., O’Neill, G. P., Liu, Q., Im, D. S., Sawyer, N., Metters, K. M., Coulombe, N., Abramovitz, M., Figueroa, D. J., Zeng, Z., Connolly, B. M., Bai, C., Austin, C. P., Chateauneau, A., Stocco, R., Greig, G. M., Kargman, S., Hooks, S. B., Hosfield, E., Williams, D. L. Jr., Ford-Hutchinson, A. W., Caskey, C. T., and Evans, J. F. (1999) Characterization of the human cysteinyl leukotriene CysLT1 receptor. Nature 399, 789–793
6. Bigsby, H. (2001) Leukotriene modifiers in pediatric asthma management. Pediatrics 107, 381–390
7. Rinaldo-Matthis, A., Wetterholm, A., Martinez Molina, D., Holm, J., Niegowski, D., Ohlson, E., Nordlund, P., Morgenstern, R., and Haeggström, J. Z. (2010) Arginine 104 is a key catalytic residue in leukotriene C₄ synthase. J. Biol. Chem. 285, 40771–40776
8. Jakobsson, P. J., Morgenstern, R., Mancini, J., Ford-Hutchinson, A., and Persson, B. (1999) Common structural features of MAPEG - A widespread superfamily of membrane associated proteins with highly divergent functions in eicosanoid and glutathione metabolism. Protein Sci. 8, 689–692
9. Jakobsson, P. J., Morgenstern, R., Mancini, J., Ford-Hutchinson, A., and Persson, B. (2000) Membrane-associated proteins in eicosanoid and glutathione metabolism (MAPEG) - A widespread protein superfamily. Am. J. Respir. Crit. Care Med. 161, S20–S24
10. Sheehan, D., Meade, G., Foley, V. M., and Dowd, C. A. (2001) Structure, function and evolution of glutathione transferases: implications for classification of non-mammalian members of an ancient enzyme superfamily. Biochem. J. 360, 1–16
11. Lundstrom, K. (2004) Structural genomics on membrane proteins: Mini review. Comb. Chem. High Throughput Screen. 7, 431–439
12. Müller, G. (2000) Towards 3D structures of G protein-coupled receptors: A multidisciplinary approach. Curr. Med. Chem. 7, 861–888
13. Ago, H., Kanaoka, Y., Irikura, D., Lam, B. K., Shimamura, T., Austen, K. F., and Miyano, M. (2007) Crystal structure of a human membrane protein involved in cysteinyl leukotriene biosynthesis. Nature 448, 609–612
14. Martínez Molina, D., Wetterholm, A., Kohl, A., McCarthy, A. A., Niegowski, D., Ohlson, E., Hammarberg, T., Eshaghi, S., Haeggström, J. Z., and Nordlund, P. (2007) Structural basis for synthesis of inflammatory mediators by human leukotriene C-4 synthase. Nature 448, 613–616
15. Sato, H., Ukita, Y., Ago, H., Irikura, D., Nisawa, A., Uneo, G., Yamamoto, M., Kanaoka, Y., Lam, B. K., Austen, K. F., and Miyano, M. (2011) The catalytic architecture of leukotriene C₄ synthase with two arginine residues. J. Biol. Chem. 286, 16392–16401
16. Sjögren, T., Nord, J., Ek, M., Johansson, P., Liu, G., and Geschwindner, S. (2013) Crystal structure of microsomal prostaglandin E-2 synthase provides insight into diversity in the MAPEG superfamily. Proc. Natl. Acad. Sci. U.S.A. 110, 3806–3811
17. Collaborative Computational Project, Number 4 (1994) The CCP4 suite: programs for protein crystallography. Acta Crystallogr. D Biol. Crystallogr. 50, 760–763
18. Kabsch, W. (2010) XDS. Acta Crystallogr. D. Biol. Crystallogr. 66, 125–132
19. Karplus, P. A., and Diederichs, K. (2012) XDS: linking crystallographic model and data quality. Science 336, 1030–1033
20. McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Sto
Leukotriene C4 Synthase in Complex with Product Analogs

roni, L. C., and Read, R. J. (2007) Phaser crystallographic software. J. Appl. Crystallogr. 40, 658 – 674
22. Murshudov, G. N., Vagin, A. A., and Dodson, E. J. (1997) Refinement of macromolecular structures by the maximum-likelihood method. Acta Crystallogr. D Biol. Crystallogr. 53, 240 – 255
23. Adams, P. D., Afonine, P. V., Bunkóczi, G., Chen, V. B., Davis, I. W., Echols, N., Headd, J. J., Hung, L. W., Kapral, G. J., Grosse-Kunstleve, R. W., McCoy, A. J., Moriarty, N. W., Oeffner, R., Read, R. J., Richardson, D. C., Richardson, J. S., Terwilliger, T. C., and Zwart, P. H. (2010) PHENIX: a comprehensive Python-based system for macromolecular structure solution. Acta Crystallogr. D Biol. Crystallogr. 66, 213 – 221
24. Emsley, P., and Cowtan, K. (2004) Coot: model-building tools for molecular graphics. Acta Crystallogr. D Biol. Crystallogr. 60, 2126 – 2132
25. Delano, W. L. (2010) The PyMOL Molecular Graphics System, version 1.3r1, Schrödinger, LLC, New York
26. Cao, J., Burke, J. E., and Dennis, E. A. (2013) Using hydrogen/deuterium exchange mass spectrometry to define the specific interactions of the phospholipase A2 superfamily with lipid substrates, inhibitors, and membranes. J. Biol. Chem. 288, 1806 – 1813
27. Smith, W. L., DeWitt, D. L., and Garavito, R. M. (2000) Cyclooxygenases: structural, cellular, and molecular biology. Annu. Rev. Biochem. 69, 145 – 182
28. Hayes, J. D., Flanagan, J. U., and Jowsey, I. R. (2005) Glutathione transferases. Annu. Rev. Pharmacol. Toxicol. 45, 51 – 88
29. Salinas, A. E., and Wong, M. G. (1999) Glutathione S-transferases - A review. Curr. Med. Chem. 6, 279 – 309