Fatty acid binding proteins stabilize leukotriene A$_4$: competition with arachidonic acid but not other lipoxygenase products

Jennifer S. Dickinson Zimmer,*,† Douglas F. Dyckes,§ David A. Bernlohr,** and Robert C. Murphy[1],*,†

Department of Pharmacology,* University of Colorado Health Sciences Center, Aurora, CO 80045; Division of Cell Biology,† Department of Pediatrics, National Jewish Medical and Research Center, Denver, CO 80206; Department of Chemistry,§ University of Colorado at Denver, Denver, CO 80217; and Department of Biochemistry, Molecular Biology, and Biophysics,** University of Minnesota, Minneapolis, MN 55455

Abstract Leukotriene A$_4$ (LTA$_4$) is a chemically reactive conjugated triene epoxide product derived from 5-lipoxygenase oxygenation of arachidonic acid. At physiological pH, this reactive compound has a half-life of less than 3 s at 37°C and $\sim$40 s at 4°C. Regardless of this aqueous instability, LTA$_4$ is an intermediate in the formation of biologically active leukotrienes, which can be formed through either intracellular or transcellular biosynthesis. Previously, epithelial fatty acid binding protein (E-FABP) present in RBL-1 cells was shown to increase the half-life of LTA$_4$ to $\sim$20 min at 4°C. Five FABPs (adipocyte FABP, intestinal FABP, E-FABP, heart/muscle FABP, and liver FABP) have now been examined and also found to increase the half-life of LTA$_4$ at 4°C to $\sim$20 min with protein present. Stabilization of LTA$_4$ was examined when arachidonic acid was present to compete with LTA$_4$ for the binding site on E-FABP. Arachidonate has an apparent higher affinity for E-FABP than LTA$_4$ and was able to completely block stabilization of the latter. When E-FABP is not saturated with arachidonate, FABP can still stabilize LTA$_4$. ■ Several lipoxygenase products, including 5-hydroxyeicosatetraenoic acid, 5,6-dihydroxyeicosatetraenoic acid, and leukotriene B$_4$, were found to have no effect on the stability of LTA$_4$ induced by E-FABP even when present at concentrations 3-fold higher than LTA$_4$.—Dickinson Zimmer, J. S., D. F. Dyckes, D. A. Bernlohr, and R. C. Murphy. **Fatty acid binding proteins stabilize leukotriene A$_4$: competition with arachidonic acid but not other lipoxygenase products.** *J. Lipid Res.* 2004. **45:** 2138–2144.

Supplementary key words half-life • transcellular biosynthesis • leukotriene biosynthesis

Leukotrienes are a family of biologically active metabolites of arachidonic acid known to play a role in a number of different pathophysiological processes. The biosynthesis of leukotrienes is initiated by the activation and translocation of cytosolic phospholipase A$_2$ (cPLA$_2$) to the nuclear envelope (1). Once cPLA$_2$ is activated, it can release arachidonic acid from the *sn*-2 position of membrane phospholipids (2). The leukotrienes are formed via the dioxygenation of arachidonic acid by the enzyme 5-lipoxygenase (5-LO) (3). This enzyme, which in resting cells is either cytosolic or nucleoplasmic, depending upon the cell type, translocates to the nuclear envelope after cell stimulation (4, 5). After translocation, 5-LO acts together with 5-LO-activating protein (FLAP), which is thought to function by presenting nonesterified arachidonic acid to 5-LO (6, 7).

After free arachidonate is presented to 5-LO, the enzyme can catalyze two separate enzymatic reactions (8). The first reaction involves the stereospecific addition of molecular oxygen to carbon-5 of arachidonic acid to form 5-(S)-hydroperoxyeicosatetraenoic acid (5-HpETE). The second reaction involves the conversion of 5-HpETE into the chemically reactive, conjugated triene epoxide leukotriene A$_4$ (LTA$_4$). LTA$_4$ is then substrate for two discrete enzymes that form the biologically active leukotrienes. LTA$_4$ hydrolase can convert LTA$_4$ into leukotriene B$_4$ (LTB$_4$), a chemotactic factor for human neutrophils, (9) and leukotriene C$_4$ synthase can convert LTA$_4$ into the cysteinyl leukotrienes (leukotrienes C$_4$, D$_4$, and E$_4$), which are myotropic agents (10). Alternatively, this epoxide intermediate can react with water rapidly at physiological pH with a half-life of less than 3 s at 37°C

Abbreviations: cPLA$_2$, cytosolic phospholipase A$_2$; diHETE, dihydroxyeicosatetraenoic acid; EET, epoxyeicosatrienoic acid; FABP, fatty acid binding protein; FLAP, 5-lipoxygenase-activating protein; HETE, hydroxyeicosatetraenoic acid; HpETE, hydroperoxyeicosatetraenoic acid; LTA$_4$, leukotriene A$_4$; LTB$_4$, leukotriene B$_4$; TEA, triethylamine; 5-LO, 5-lipoxygenase.

[1] To whom correspondence should be addressed.
  e-mail: robert.murphy@uchsc.edu

(11). This chemical reaction results in the formation of a carbocation intermediate and a number of products, including inactive LTB_4 isomers such as 5,12-dihydroxyeicosatetraenoic acid (5,12-diHETE) and 5,6-diHETE (12).

We recently discovered that in RBL-1 cells, a rat basophilic leukemia cell line often used to study leukotriene biosynthesis and biochemistry, a cytosolic protein can stabilize LTA_4. This protein was identified as the epithelial fatty acid binding protein (E-FABP) (13). There is increasing evidence that the FABP family may be involved in the intracellular trafficking of eicosanoid lipid mediators. Recent work has shown that E-FABP can bind to the 5-LO products 5-HpETE and 5-hydroxyeicosatetraenoic acid (5-HETE) with reasonably high affinity (14). Epoxyeicosatrienoic acids (EETs), products of cytochrome P450 metabolism of arachidonic acid, have also been found to be ligands for other FABPs (15). Other recent investigations have shown that EETs can be bound to heart FABP or intestinal FABP, remaining protected from hydrolysis when soluble epoxide hydrolase is added to the buffer (16).

FABPs are a family of low molecular mass ($\sim$15 kDa) proteins that have high binding affinity for various fatty acids (17). The members of this family have between 20% and 70% sequence identity; however, they all share similar tertiary structure: 10 antiparallel β-strands linked by hydrogen bonds to form a β-barrel (18). The FABP family has long been studied for its involvement in fatty acid transport in a number of different tissues (19). This protein family has been suggested to be involved in the solubilization of fatty acids in the aqueous environment within the cytoplasm and in the facilitation of transport of fatty acids into the cell by sequestering free fatty acids in the cytosol (20).

Our recent finding that E-FABP was a LTA_4-stabilizing protein in RBL-1 cells led us to investigate the interaction between E-FABP and other FABP family members with LTA_4 in more depth. In addition, the binding between E-FABP and LTA_4 was examined further by using other fatty acids and lipoxygenase products as competitors for the LTA_4 binding site on E-FABP.

MATERIALS AND METHODS

Materials

All eicosanoids were obtained from Cayman Chemical Co. (Ann Arbor, MI). LTA_4-free acid was prepared as previously described (21). Histidine-tagged FABPs were prepared as previously described (22). These His-tagged FABPs included mouse adipocyte FABP (mA-FABP), mouse epithelial FABP (mE-FABP), human heart/muscle FABP (hH/M-FABP), rat intestinal FABP (rI-FABP), and rat liver FABP (rL-FABP). In addition, non-histidine-tagged rat epithelial FABP (rE-FABP) was prepared as previously described (14). Triethylamine was purchased from Aldrich Chemical Co. (Milwaukee, WI). All other solvents and reagents were HPLC grade and purchased from Fisher Scientific (Fair Lawn, NJ).

LTA_4 stabilization assays

To determine the chemical half-life of LTA_4, 500 ng of the free acid was added to 250 μl of buffer or protein solution and incubated at 4°C; the resulting concentrations of organic solvents in the incubation mixture were 0.2% methanol and 0.16% acetone.

Aliquots (50 μl) were removed at various time points between 2 and 30 min and added to ethanol (100 μl) containing 100 ng of an internal standard, which was either 15-oxo-ETE or 20-trifluoro-LTA_4. This sample was centrifuged and then added to 150 μl of 10 mM triethylamine (TEA) to bring it up to initial conditions for HPLC. A reverse-phase XTerra MS column (2.1 × 50 mm, 3.5 μm C18; Waters Corp., Milford, MA) was used at a flow rate of 200 μl/min with a linear gradient using mobile phase A consisting of 10 mM triethylamine at pH 11 and mobile phase B consisting of acetonitrile-methanol (65:35, v/v) containing 10 mM triethylamine (TEA). The gradient started at 30% B for initial conditions and increased to 80% B in 5 min. These samples were analyzed using either an in-line photodiode array or a triple quadrupole mass spectrometer (described below). In either case, the log ratio of the peak area of LTA_4 to the peak area of its internal standard versus time was plotted and the half-life was calculated using the slope of the resulting line.

Assessment of stabilization capacity (units of protein activity) was used to determine differences in protein stabilization during competition assays. For these assays, LTA_4 free acid (100 ng) was added to either buffer or protein fraction (50 μl). For competition assays, various concentrations of the competitor lipid were mixed with LTA_4 before addition to the protein or buffer. Each sample was allowed to incubate at 4°C for 20 min, then ethanol (100 μl) containing 100 ng of 15-oxo-ETE was added. The sample was brought to initial HPLC conditions by the addition of 150 μl of 10 mM TEA, and the ultraviolet absorbance (280 nm) from LTA_4 and 15-oxo-ETE was determined at their corresponding retention times. Units of protein activity were defined as 10 times the ratio of milliabsorbance units of LTA_4/milliabsorbance units of 15-oxo-ETE after the subtraction of the same ratio measured in buffer in the absence of protein. It is difficult to assess the actual amount of LTA_4 that was available for association with FABP in this assay because of two complicating factors. The first problem is the chemical reactivity of this eicosanoid, which immediately begins to degrade when added to an aqueous solution of the FABP. Immediate mixing was used to minimize this problem. Second, it was found that $\sim$6 ± 2% of the added LTA_4 became associated with the polypropylene reaction tube because of the extreme lipophilicity of LTA_4 (data not shown). However, based on the quantity of LTA_4 eluting from the HPLC column in this stabilization assay (as assessed from a standard curve relative to the 15-oxo-ETE internal standard), a value reported as 300 units/mg corresponds to $\sim$30% recovery of the initially added LTA_4.

Displacement of bound arachidonic acid

To preload E-FABP with arachidonic acid, the protein was incubated for 15–30 min with 20 μM arachidonic acid. The protein was then subjected to size-exclusion chromatography on a desalting column (Econo-Pac® 10DG Disposable Desalting Column; Bio-Rad Laboratories, Hercules, CA) to remove the free arachidonate from the buffer solution and then tested for stabilizing activity. LTA_4 (9 μM) was then added to the arachidonate-preloaded E-FABP (4.5 μM), and the units of stabilization were determined as described above. The extent of arachidonate remaining associated with the E-FABP was assessed by repeating the binding and gel-filtration steps using [^{3}H]arachidonate of a known specific activity and determining the bound arachidonate in the eluted E-FABP by scintillation counting.

Mass spectrometry

For the LTA_4 half-life experiments analyzed by mass spectrometry, the HPLC effluent was introduced into a Sciex API-3000 (PE-Sciex, Thornhill, Ontario, Canada), and the samples were analyzed as previously described (23).

RESULTS

Stabilization of LTA$_4$ by FABP family members

Previously, we had reported that the E-FABP, also known as the keratinocyte lipid binding protein, was present in RBL-1 cells and could protect LTA$_4$ from rapid hydrolysis with water (13). To ascertain whether this phenomenon was specific for E-FABP, we tested the ability of FABPs from various tissues and species to carry out this same function (**Fig. 1**). Most of the FABPs that we tested were His-tagged, so any interference by the His tag on the process of LTA$_4$ stabilization was investigated. The rE-FABP (4.5 μM) and the His-tagged mE-FABP (4.5 μM), which have 92% sequence identity, were tested for their ability to increase the half-life of LTA$_4$ (9 μM) (Fig. 1). There was no difference between the half-life of LTA$_4$ incubated with the His-tagged mE-FABP (17.2 $\pm$ 0.76 min) or with rE-FABP (14.9 $\pm$ 2.0 min); both proteins were able to increase the half-life of LTA$_4$ by at least 25-fold over buffer alone, which contained an identical amount of ethanol and acetone added as the vehicle to dissolve the free acid LTA$_4$ used for these half-life assays (Fig. 1).

The other proteins tested were also His-tagged, including the mA-FABP, the mE-FABP, the hH/M-FABP, the rI-FABP, and the rL-FABP. When the concentration of FABP was held constant at 4.5 μM, the half-life of LTA$_4$ was examined at 4°C in the buffer solution (Fig. 1). Under these conditions, the half-life of LTA$_4$ in buffer (in the absence of any FABP) was less than 45 s; however, all of the FABPs were able to stabilize LTA$_4$ to a half-life of greater than 10 min. The epithelial FABP from both mouse and rat seem to have a slightly greater ability to stabilize LTA$_4$, but this difference was not statistically significant.

In separate experiments, LTA$_4$ (9 μM) was added to horse myoglobin (4.5 μM), which has a molecular mass of 16,900 Da, similar to the FABPs, but a completely dissimilar tertiary structure. There was no difference in the half-life of LTA$_4$ in the presence of this protein relative to buffer controls (Fig. 1), supporting the hypothesis that the stabilization effect of E-FABP was specific rather than a nonspecific protein effect.

Competition assays

To examine the binding between LTA$_4$ and E-FABP in further detail, competition assays between LTA$_4$ and other lipids were performed. These assays were carried out with target lipids and LTA$_4$ at 9 μM added together to E-FABP at 4.5 μM. Arachidonic acid has been reported to be one of the highest affinity ligands for E-FABP, with a dissociation constant of 318 $\pm$ 14 nM (14).

In those experiments in which arachidonate (15 μM) was in excess of LTA$_4$ (9 μM) in the competition experiment, there was a complete loss of any evidence of LTA$_4$ stabilization (**Fig. 2**). When arachidonate (7.5 μM) was similar to the concentration of LTA$_4$ (9 μM), there was also little suggestion that any stabilization of LTA$_4$ occurred. Only when the arachidonate was reduced to less than 3.8 μM was there any stabilization of LTA$_4$, suggesting that LTA$_4$ competed very poorly for E-FABP binding with arachidonic acid but that when a sufficient number of empty sites remained, LTA$_4$ could enter the binding cavity and be sequestered in a region protected from the aqueous environment.

Many studies of fatty acid binding to these FABP proteins use techniques in which equilibrium conditions exist to measure a dissociation constant. However, little is known about the rates at which fatty acids enter or leave the binding cavity. Nevertheless, the investigation of LTA$_4$ stabilization by FABP is in fact somewhat different from such measurements and more likely follows the competition of this triene epoxide with other fatty acids such as arachidonate for entry into the binding cavity. Although the arachidonate can freely enter and leave in a dynamic manner that
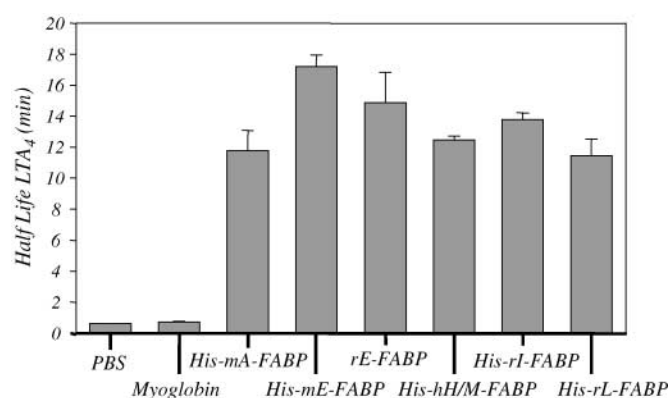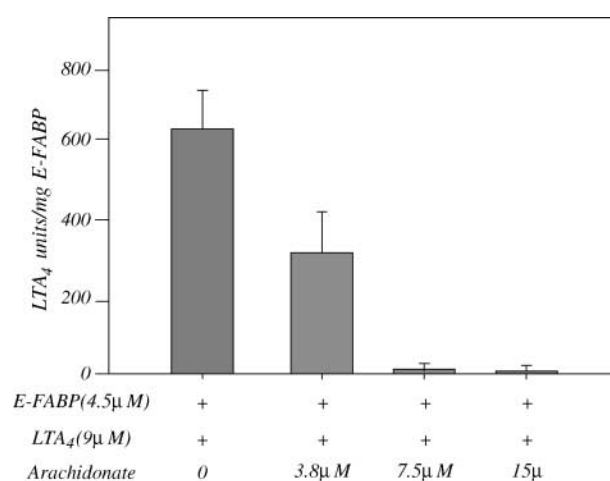

Fig. 1. Stabilization of leukotriene A$_4$ (LTA$_4$) by various members of the fatty acid binding protein (FABP) family as measured by an increase in the half-life of LTA$_4$ (9 μM) by 4.5 μM FABP and by 4.5 μM horse myoglobin. The proteins tested were non-His-tagged recombinant rat epithelial FABP (rE-FABP) and the His-tagged recombinant proteins mouse epithelial FABP (His-mE-FABP), mouse adipocyte FABP (His-mA-FABP), human heart/muscle FABP (His-hH/M-FABP), rat intestinal FABP (His-rI-FABP), and rat liver FABP (His-rL-FABP). Results are reported as averages of four experiments $\pm$ SEM.

Fig. 2. Stabilization of LTA$_4$ (9 μM) by 4.5 μM rE-FABP in the presence of increasing concentrations of arachidonic acid (3.8, 7.5, and 15 μM) measured by the amount of LTA$_4$ remaining after 20 min at 4°C in a standard assay (see Materials and Methods). Results are reported as averages of four experiments $\pm$ SEM.

can be estimated by an equilibrium binding constant, once LTA_4 leaves the protective binding cavity it would be rapidly hydrolyzed by water to 6-*trans*-LTB_4, 6-*trans*-12-epi-LTB_4, and several 5,6-diHETEs. Therefore, a slightly different study was performed in which an initial equilibrium of arachidonic acid with E-FABP was established only to be followed by the addition of LTA_4. For this study, arachidonate (20 μM) was incubated on ice with E-FABP for 30 min, then the free arachidonic acid was removed by a rapid gel-filtration column. Based upon results from separate experiments in which radiolabeled arachidonate was added as a tracer, the concentration of arachidonate associated in the gel filtration fraction containing E-FABP was only slightly more than half of the assumed concentration of protein, equivalent to 2.3 μM. This suggested that rapid dissociation of the arachidonate had occurred while on the column, which became efficiently trapped by the gel-filtration column (**Fig. 3**). In fact, the gel-associated radiolabeled arachidonate was not efficiently eluted until the column was injected with fatty acid-free BSA. When the gel-separated E-FABP (4.5 μM) containing 2.3 μM arachidonate was incubated with 9 μM LTA_4, the LTA_4 was stabilized to 330 ± 7 LTA_4 units/mg E-FABP (Fig. 3), a value

similar to that seen when E-FABP (4.5 μM) was mixed with a mixture of 3.8 μM arachidonate simultaneously with 9 μM LTA_4 (Fig. 2).

The striking stabilization of LTA_4 by various FABP proteins suggested that this family could play a central and previously unrecognized role in leukotriene biosynthesis within the cell. Therefore, various 5-LO products were examined as potential binding partners to assess whether or not these products could reduce the extent of LTA_4 stability, as did arachidonate (**Fig. 4**). The 5-LO product 5(*S*)-HETE had been previously shown to have rather low affinity for E-FABP (1,560 ± 115 nM) (14), but other LTA_4 hydrolysis products, such as 5,6-diHETE and leukotriene B_4 (LTB_4), had not been previously examined. The stability of LTA_4 induced by E-FABP was only slightly decreased by 5-HETE, and then only when present at relatively high concentrations of 10 or 30 μM. In sharp contrast, arachidonate at 15 μM completely abolished the LTA_4 stability in this same series of experiments. When either 5,6-diHETE or LTB_4 was added to this competition assay for LTA_4 stability, there was no evidence for any effect even when a greater than 3-fold higher molar ratio of each dihydroxy-eicosanoid was used (Fig. 4). LTA_4 remained highly stable in these assays. These results suggest that the products of LTA_4 that result from either enzymatic or nonenzymatic hydrolysis of the triene epoxide do not effectively compete for the FABP cavity that stabilizes LTA_4.

DISCUSSION

Stabilization by FABPs

FABPs are found in the cytosol of most cells; however, the tissue distribution of the FABP family members varies (24). Only A-FABP and E-FABP have been shown to be expressed in cells of the myeloid lineage, which can produce LTA_4 (25, 26), and perhaps these FABPs play a role in facilitating the distribution of LTA_4 within these cells. The other FABP family members could still play some role in
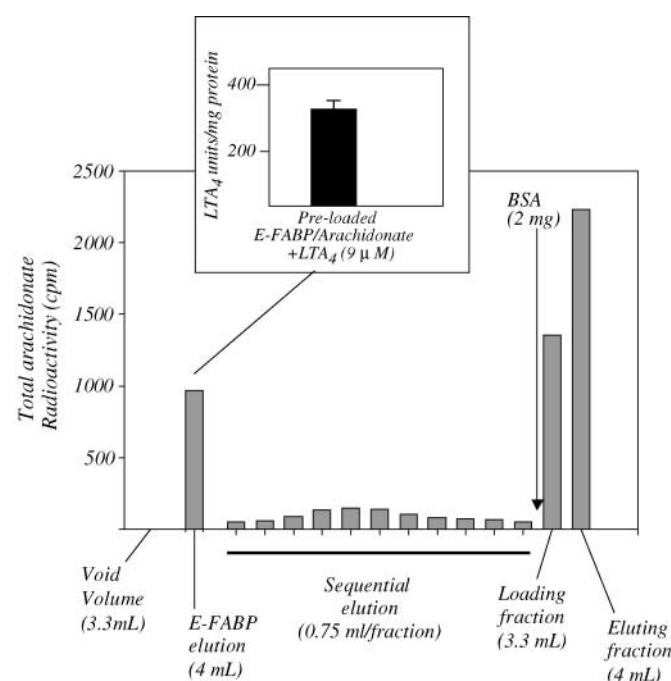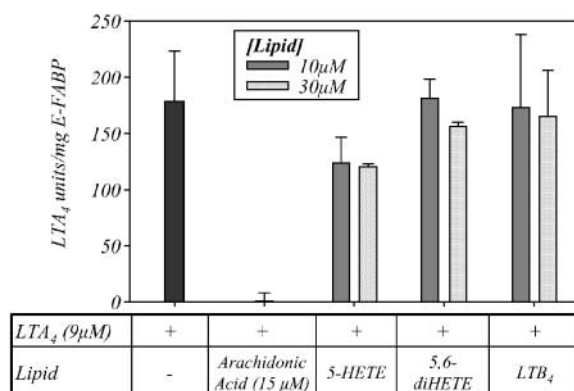

Fig. 3. Analysis of rE-FABP preequilibrated with [^{3}H]arachidonate. Separation of unbound arachidonate was carried out by a gel-filtration column using phosphate-buffered saline elution, with collection of void column fraction (3.3 ml), total protein fraction (4 ml), or 1 min fraction (0.75 ml). After washing with a total of two column volumes and collecting sequential 0.75 ml fractions, 2 mg of BSA was added (1 mg/ml) to the column followed by elution of the corresponding void volume and protein fraction as above. Aliquots of each fraction were taken for the determination of radioactivity content by scintillation counting. The inset shows results from the assay of LTA_4 stabilization of the preequilibrated and gel filtration-purified E-FABP (4.5 μM) calculated to contain 2.3 μM arachidonate from the radioactivity tracer studies. Results are reported as the average of four experiments ± SEM.

Fig. 4. Competition for E-FABP (4.5 μM) binding between LTA_4 (9 μM) and various eicosanoids at concentrations of either 10 or 30 μM as determined by LTA_4 stability. Experiments with arachidonate were carried out at 15 μM. Results are reported as averages of four experiments ± SEM. diHETE, dihydroxyeicosatetraenoic acid; HETE, hydroxyeicosatetraenoic acid; LTB_4, leukotriene B_4.

the biosynthesis of leukotrienes by stabilizing LTA$_4$ in acceptor cells for transcellular biosynthesis. Even though many of the details of the process of transcellular biosynthesis have yet to be elucidated, it is now well established that the LTA$_4$ produced in the neutrophil or other cell types, such as the monocyte, can appear within other cell types in which conversion into the biologically active leukotrienes takes place (27). Hepatocytes, which express L-FABP (28), have been shown to be able to synthesize cysteinyl leukotrienes when they are provided exogenous LTA$_4$ or when they are coincubated with monocyte-derived Kupffer cells and stimulated (29, 30). Aortic endothelial cells, which express the heart or muscle FABP (31), can also convert either exogenous or neutrophil-derived LTA$_4$ into leukotriene C$_4$ (32, 33).

Even though the primary amino acid sequences of the members of the FABP family differ extensively, all of the family members share a similar tertiary structure, which consists of 10 antiparallel β-strands forming a β-barrel (18). This β-barrel structure forms an internal, water-filled cavity that differs widely among the family members and that accounts for the majority of the sequence differences between the various family members (34). To prolong the half-life of LTA$_4$, the FABP would need to protect the acid-labile epoxide from hydrolysis by water by binding LTA$_4$ in a manner that would place the epoxide in a hydrophobic region of the binding pocket. Because of the differences in the binding pocket amino acids, it is somewhat surprising that every member of the FABP family that was tested was able to stabilize the half life of LTA$_4$ in a similar manner, but this suggests that the conjugated triene and epoxide moiety of LTA$_4$ is held in a region not readily accessible to water within the binding cavity. It should be noted that for each FABP evaluated, X-ray crystal structures with bound fatty acids have been determined; these indicate that for each species, the bound lipid is held in a structurally ordered environment in a region with crystallographically defined water molecules. As such, the exclusion of disordered water from the region surrounding the epoxide may be an inherent general property of FABPs as a component of their lipid binding character.

Competition assays

Although some of the LTA$_4$ produced by 5-LO could be bound by FABPs and protected from hydrolysis, the remaining unbound LTA$_4$ would be hydrolyzed into its major nonenzymatic products, 5,6-diHETE and the 6-*trans*-LTB$_4$ isomers (also called 5,12-diHETEs). These downstream metabolites of LTA$_4$, including the enzymatic product LTB$_4$ and the nonenzymatic hydrolysis product 5,6-diHETE, were not able to inhibit the stabilization of LTA$_4$ by E-FABP even at high concentrations (Fig. 4). If LTA$_4$ were being transported through the cytosol to LTA$_4$ hydrolase by a FABP, neither the LTB$_4$ produced by this enzyme nor the nonenzymatic hydrolysis products of LTA$_4$ would likely compete for binding with the LTA$_4$. The only lipoxygenase product that could compete with LTA$_4$ for its binding site on E-FABP was 5-HETE (Fig. 4); however, this compound only decreased the stabilization of LTA$_4$ by 33%, and even then only when at very high concentrations.

The concentration of free arachidonic acid within cells is tightly controlled by restricting the release of arachidonate and also by rapid reesterification of free arachidonate into the phospholipids (35, 36). When cells are stimulated, for example, by an influx of free calcium ions, cPLA$_2$ cleaves arachidonic acid from the phospholipids, creating high localized concentrations of free arachidonate (1). Under these conditions, 5-LO is also stimulated, so LTA$_4$ is also produced within the cell (37). In those ex-
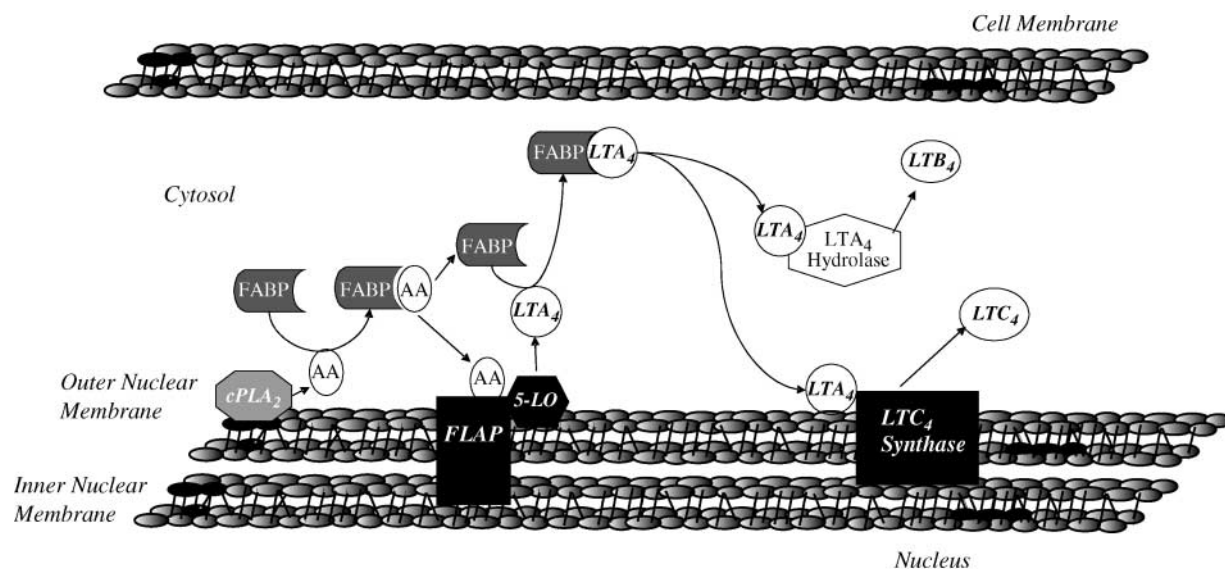

Fig. 5. Schematic diagram of the proposed role of FABP in leukotriene biosynthesis. The FABP picks up free arachidonate (AA) and delivers it to 5-lipoxygenase-activating protein (FLAP), which can present it to 5-lipoxygenase (5-LO). 5-LO then converts arachidonate into LTA$_4$, which can be picked up by the newly unoccupied FABP and delivered to the biosynthetic enzymes for the bioactive leukotrienes. cPLA$_2$, cytosolic phospholipase A$_2$; LTC$_4$, leukotriene C$_4$.

periments in which arachidonate was added at the same time, it was able to completely abolish any stabilization of LTA_4 (Fig. 2). One possible explanation for this effect of arachidonate could be the highly lipophilic character of both arachidonate and LTA_4, as mentioned above. An association complex could be formed that would prevent LTA_4 from entering the FABP cavity but still not sufficiently protecting LTA_4 from the aqueous environment, so that hydrolysis occurred. However, these experiments suggest that the capture of LTA_4 is independent from any dynamic association of arachidonate or movement of this polyunsaturated fatty acid in and out of the FABP binding cavity and only requires the availability of an empty site in proximity to LTA_4 to enable capture. In these competitive assays in which E-FABP was initially equilibrated with arachidonic acid before the addition of LTA_4, the separation of free arachidonate from the E-FABP resulted in a preparation containing only 2.3 μM arachidonate and 4.5 μM E-FABP. Using the previously published dissociation constant (14), one can estimate ~40% E-FABP containing arachidonate in an association complex with 60% empty E-FABP lipid binding sites available with which LTA_4 could quickly associate. The level of stabilization of LTA_4 by this preparation (Fig. 3) was very similar to that when 3.75 μM arachidonate and 4.5 μM E-FABP were incubated with LTA_4 (Fig. 2). For these latter conditions, the amount of arachidonate-associated E-FABP could be calculated as 66%, leaving 33% available to quickly associate with LTA_4.

These data suggest that the LTA_4 produced when a cell is stimulated could be captured by the FABP that had lost the bound arachidonate (or any other fatty acid) within the binding site of the FABP and then be stabilized for subsequent biochemical processing (**Fig. 5**).

FABPs appear to have a high binding affinity for both LTA_4 and arachidonic acid, although the true binding affinity for LTA_4 is difficult, if not impossible, to measure by the techniques used here. This protein family could aid in the production of bioactive leukotrienes, possibly via the following model (Fig. 5). Upon cell stimulation, $cPLA_2$ and 5-LO are translocated to the nuclear envelope. Active $cPLA_2$ would release arachidonic acid from the membrane phospholipids, and this arachidonate would be bound by a FABP and transported to the 5-LO/FLAP protein complex, in which the arachidonic acid could be presented to 5-LO by FLAP, thus leaving the FABP binding pocket empty. 5-LO would then convert the arachidonate into LTA_4, which could be picked up by the newly emptied FABP. This LTA_4-bound FABP would then be the vehicle delivering this epoxide intermediate to cytosolic LTA_4 hydrolase for conversion to LTB_4. The LTA_4/FABP complex could also protect the LTA_4 long enough so that it could participate in transcellular metabolism by presenting LTA_4 to the plasma membrane compartment.

In conclusion, six FABPs were shown to have the ability to stabilize LTA_4 to a half-life ~20 times greater than that in buffer alone. This chemically unstable intermediate was further shown to be able to compete effectively for FABP binding with various lipoxygenase products. Although the role of these FABPs in leukotriene biosynthesis has not been widely recognized, it is possible that they can play a role in assisting the distribution of the reactive chemical intermediate, LTA_4, throughout the cytosol, where it can be enzymatically converted into biologically active leukotrienes. It is also possible that FABPs could be important in stabilizing LTA_4 transferred from one cell to another, resulting in the transcellular biosynthesis of bioactive leukotrienes in those cells that lack 5-LO but express one member of this family of important lipid binding proteins.

REFERENCES

1. Gijón, M. A., and C. C. Leslie. 1999. Regulation of arachidonic acid release and cytosolic phospholipase A_2 activation. *J. Leukoc. Biol.* **65:** 330–336.
2. Dennis, E. A. 2000. Phospholipase A_2 in eicosanoid generation. *Am. J. Respir. Crit. Care Med.* **161 (Suppl.):** 32–35.
3. Ford-Hutchinson, A. W., M. Gresser, and R. N. Young. 1994. 5-Lipoxygenase. *Annu. Rev. Biochem.* **63:** 383–417.
4. Brock, T. G., R. Paine III, and M. Peters-Golden. 1994. Localization of 5-lipoxygenase to the nucleus of unstimulated rat basophilic leukemia cells. *J. Biol. Chem.* **269:** 22059–22066.
5. Rouzer, C., and S. Kargman. 1988. Translocation of 5-lipoxygenase to the membrane in human leukocytes challenged with ionophore A23187. *J. Biol. Chem.* **263:** 10980–10988.
6. Miller, D. K., J. W. Gillard, P. J. Vickers, S. Sadowski, C. Léveillé, J. A. Mancini, P. Charleson, R. A. F. Dixon, A. W. Ford-Hutchinson, R. Fortin, J. Y. Gauthier, J. Rodkey, R. Rosen, C. Rouzer, I. S. Sigal, C. D. Strader, and J. F. Evans. 1990. Identification and isolation of a membrane protein necessary for leukotriene production. *Nature.* **343:** 278–281.
7. Mancini, J. A., M. Abramovitz, M. E. Cox, E. Wong, S. Charleson, H. Perrier, Z. Wang, P. Prasit, and P. J. Vickers. 1993. 5-Lipoxygenase-activating protein is an arachidonate binding protein. *FEBS Lett.* **318:** 277–281.
8. Shimizu, T., O. Rådmark, and B. Samuelsson. 1984. Enzyme with dual lipoxygenase activities catalyzes leukotriene A4 synthesis from arachidonic acid. *Proc. Natl. Acad. Sci. USA.* **81:** 689–693.
9. Haeggström, J., F. Kull, P. C. Rudberg, F. Tholander, and M. M. G. M. Thunnissen. 2002. Leukotriene A_4 hydrolase. *Prostaglandins Other Lipid Med.* **68–69:** 495–510.
10. Lam, B. K. 2003. Leukotriene C_4 synthase. *Prostaglandins Leukot. Essent. Fatty Acids.* **69:** 111–116.
11. Fitzpatrick, F. A., D. R. Morton, and M. A. Wynalda. 1982. Albumin stabilizes leukotriene A_4. *J. Biol. Chem.* **257:** 4680–4683.
12. Borgeat, P., and B. Samuelsson. 1979. Arachidonic acid metabolism in polymorphonuclear leukocytes: unstable intermediate in formation of dihydroxy acids. *Proc. Natl. Acad. Sci. USA.* **76:** 3213–3217.
13. Dickinson Zimmer, J. S., D. R. Voelker, D. A. Bernlohr, and R. C. Murphy. 2004. Stabilization of leukotriene A_4 by epithelial fatty acid binding protein in the rat basophilic leukemia cell. *J. Biol. Chem.* **279:** 7420–7426.
14. Kane, C. D., N. Ribarik Coe, B. Vanlandingham, P. Krieg, and D. A. Bernlohr. 1996. Expression, purification, and ligand-binding analysis of recombinant keratinocyte lipid-binding protein (MAL-1), an intracellular lipid-binding protein overexpressed in neoplastic skin cells. *Biochemistry.* **35:** 2894–2900.
15. Widstrom, R. L., A. W. Norris, and A. A. Spector. 2001. Binding of cytochrome P450 and lipoxygenase pathway products by heart fatty acid-binding protein. *Biochemistry.* **40:** 1070–1076.
16. Widstrom, R. L., A. W. Norris, J. Van Der Veer, and A. A. Spector. 2003. Fatty acid-binding proteins inhibit hydration of epoxyeicosatrienoic acids by soluble epoxide hydrolase. *Biochemistry.* **42:** 11762–11767.

17. Storch, J., and A. E. A. Thumser. 2000. The fatty acid transport function of fatty acid-binding proteins. *Biochim. Biophys. Acta.* **1486:** 28–44.

18. Sacchettini, J. C., J. I. Gordon, and L. J. Banaszak. 1989. Crystal structure of rat intestinal fatty-acid-binding protein. Refinement and analysis of the *Escherichia coli*-derived protein with bound palmitate. *J. Mol. Biol.* **208:** 327–339.

19. Zimmerman, A. W., H. T. B. van Moerkerk, and J. H. Veerkamp. 2001. Ligand specificity and conformational stability of human fatty acid-binding proteins. *Int. J. Biochem. Cell Biol.* **33:** 865–876.

20. Glatz, J. F. C., J. J. F. P. Luiken, M. van Bilsen, and G. J. van der Vusse. 2002. Cellular lipid binding proteins as facilitators and regulators of lipid metabolism. *Mol. Cell. Biochem.* **239:** 3–7.

21. Carrier, D. J., T. Bogri, G. P. Cosentino, I. Guse, S. Rakhit, and K. Singh. 1988. HPLC studies on leukotriene A_4 obtained from hydrolysis of its methyl ester. *Prostaglandins Leukot. Essent. Fatty Acids.* **34:** 27–30.

22. Jenkins-Kruchten, A. E., A. Bennars-Eiden, J. R. Ross, W. J. Shen, F. B. Kraemer, and D. A. Bernlohr. 2003. Fatty acid binding protein-hormone sensitive lipase interaction: fatty acid dependence on binding. *J. Biol. Chem.* **278:** 47636–47643.

23. Dickinson, J. S., and R. C. Murphy. 2002. Mass spectrometric analysis of leukotriene A_4 and other chemically reactive metabolites of arachidonic acid. *J. Am. Soc. Mass Spectrom.* **13:** 1227–1234.

24. Hertzel, A. V., and D. A. Bernlohr. 2000. The mammalian fatty acid-binding protein multigene family: molecular and genetic insights into function. *Trends Endocrinol. Metab.* **11:** 175–180.

25. Guthmann, F., C. Hohoff, H. Fechner, P. Humber, T. Börchers, F. Spener, and B. Rüstow. 1998. Expression of fatty-acid-binding-proteins in cells involved in lung-specific lipid metabolism. *Eur. J. Biochem.* **253:** 430–436.

26. Owada, Y., S. A. Abdelwahab, R. Suzuki, H. Iwasa, and H. Sakagami. 2001. Localization of epidermal-type fatty acid binding protein in alveolar macrophages and some alveolar type II epithelial cells in mouse lung. *Histochem. J.* **33:** 453–457.

27. Bigby, T. D., and N. Meslier. 1989. Transcellular lipoxygenase metabolism between monocytes and platelets. *J. Immunol.* **143:** 1948–1954.

28. Ockner, R. K., J. A. Manning, and J. P. Kane. 1982. Fatty acid binding protein: isolation from rat liver, characterization and immunochemical quantification. *J. Biol. Chem.* **257:** 7872–7878.

29. Fukai, F., Y. Suzuki, H. Ohtaki, and T. Katayama. 1993. Rat hepatocytes generate peptide leukotrienes from leukotriene A_4. *Arch. Biochem. Biophys.* **305:** 378–384.

30. Fukai, F., Y. Suzuki, Y. Nishizawa, and T. Katayama. 1996. Transcellular biosynthesis of cysteinyl leukotrienes by Kupffer cell-hepatocyte cooperation in rat liver. *Cell Biol. Int.* **20:** 423–428.

31. Antohe, F., D. Popov, L. Radulescu, N. Simionescu, T. Borchers, F. Spener, and M. Simionescu. 1998. Heart microvessels and aortic endothelial cells express the 15kDa heart-type fatty acid-binding proteins. *Eur. J. Cell Biol.* **76:** 102–109.

32. Feinmark, S. J., and P. J. Cannon. 1986. Endothelial cell leukotriene C_4 synthase results from intercellular transfer of leukotriene A_4 synthesized by polymorphonuclear leukocytes. *J. Biol. Chem.* **264:** 16466–16472.

33. Sala, A., G. Rossoni, F. Berti, C. Buccellati, A. Bonazzi, J. Maclouf, and G. Folco. 2000. Monoclonal anti-CD18 antibody prevents transcellular biosynthesis of cysteinyl leukotrienes in vitro and in vivo and protects against leukotriene-dependent increase in coronary vascular resistance and myocardial stiffness. *Circulation.* **101:** 1436–1440.

34. Coe, N. R., and D. A. Bernlohr. 1998. Physiological properties and functions of intracellular fatty acid-binding proteins. *Biochim. Biophys. Acta.* **1391:** 287–306.

35. Alonso, F., P. M. Henson, and C. C. Leslie. 1986. A cytosolic phospholipase in human neutrophils that hydrolyzes arachidonoyl-containing phosphatidylcholine. *Biochim. Biophys. Acta.* **878:** 273–280.

36. Chilton, F. H., and R. C. Murphy. 1986. Remodeling of arachidonate-containing phosphoglycerides within the human neutrophil. *J. Biol. Chem.* **261:** 7771–7777.

37. Wong, A., M. N. Cook, J. P. Foley, H. M. Sarau, and S. M. Hwang. 1991. Influx of extracellular calcium is required for the membrane translocation of 5-lipoxygenase and leukotriene synthesis. *Biochemistry.* **30:** 9346–9354.