Leukotrienes are a family of biologically active metabolites of arachidonic acid known to play important roles in multiple physiological and pathophysiological processes by acting as lipid mediators through specific G protein-coupled receptors (1, 2). The biosynthesis of these 20 carbon fatty acids is regulated by activating protein (11) and arachidonic acid, which is released by cytosolic phospholipase A₂ (12), the biosynthesis of LTA₄ takes place. Studies of the fate of LTA₄ produced within the human neutrophil revealed that greater than 50% of this lipid generated after cell activation is released to participate in the process of transcellular metabolism (13). Although many of the details of this process are unclear, it is now established that LTA₄ produced in the neutrophil can appear within other cell types that express synthetic enzymes for the biologically active leukotrienes. For example, erythrocytes, which express LTA₄ hydrolase but do not express 5-lipoxygenase, have been shown to convert LTA₄ into LTB₄ (14). Endothelial cells and platelets, which express LTC₄ synthase but have no 5-lipoxygenase, have been shown to convert LTA₄ derived from the neutrophil into LTC₄ (15, 16). Because of the chemical instability of LTA₄, it is clear that some mechanism must protect LTA₄ from exposure to water, preventing the nonenzymatic hydrolysis during transit between cells.

Both the chemical instability and the extent of transcellular
biosynthesis taking place in multiple cellular compartments sug-
gest that LTA₄ is stabilized by binding to an intracellular protein that protects LTA₄ from water. Previous work has shown that serum albumin from various species can increase the half-life of LTA₄ to more than 20 min at concentrations that are found in plasma (7). However, intracellular proteins that function to protect LTA₄ from hydrolysis have not been identi-
fied. The purpose of our study was to critically test for the presence of LTA₄-stabilizing proteins within RBL cells and identify the stabilizing proteins. RBL-1 cells were chosen for these studies because this cell line expresses 5-lipoxygenase and therefore is likely to have a protein that functions in lipid stabilization (18). In addition, leukotriene biosynthesis has been extensively studied and characterized in these cells (19). Our findings now identify epithelial fatty acid-binding protein as an important molecule for stabilizing LTA₄ in these cells.

EXPERIMENTAL PROCEDURES

Materials—LTA₄ ethyl ester was a generous gift from Dr. Joseph Mancini at Merck-Frosst Canada (Pointe-Claire, Canada). All other eicosanoids were obtained from the Cayman Chemical Company (Ann Arbor, MI). LTA₄-free acid was prepared as previously described (20). Anti-epithelial fatty acid-binding protein (anti-E-FABP) and anti-adipocyte fatty acid-binding protein (anti-A-FABP) antibodies were prepared as previously described (21). Nonspecific rabbit IgG and trieth-
olamine were purchased from Aldrich. All other solvents and reagents were HPLC grade and were purchased from Fisher.

Cytosol Preparation—Rat basophilic leukemia cells (RBL-1) were cultured at the National Cell Culture Center (Minneapolis, MN). The cells were grown in suspension to a density between 0.85 × 10⁶ and 1.5 × 10⁸ cells/ml in Joklik’s medium supplemented with 10% fetal bovine serum. The cells were harvested by centrifugation at 2500 × g. The pellet was resuspended and washed twice with phosphate-buffered saline. The final cell pellet was snap frozen and stored at −70 °C until use. Cytosol preparation was performed as previously described (22).

Protein Purification—Ammonium sulfate precipitation was per-
formed as previously described (23) to remove any proteins insoluble at 30% saturation (w/v) or soluble above 75% saturation (w/v). The pellet from this centrifugation was resuspended in a total volume of 2 ml of 50 mM NaOAc, pH 5.0, and injected onto a Superose 12 prep grade column (16 × 30 mm) (Amersham Biosciences) at a flow rate of 1 ml/min. A mixture of known proteins was chromatographed under the same con-
ditions to create a molecular mass calibration curve. The active frac-
tions from the size exclusion chromatography were pooled and exchanged into 25 mM Tris, pH 7.2, using a desalting column (Econo-Pac® CM, 5-ml bed volume; Bio-Rad) with a flow rate of 2 ml/min and a gradient from 0 to 1 M NaCl in 25 min. The active fraction in this purification step was 15-oxy-ETE (100 ng) was added to either buffer or protein fraction (50 μl). Each sample was allowed to incubate at 4 °C for 20 min, and then ethanolic (100 μl) containing 15-oxy-ETE (100 ng) was added. The sample was brought to initial HPLC conditions by the addition of 150 μl of 10 μM triethyl-
amine, and the UV absorbance (280 nm) from LTA₄ and 15-oxy-ETE was determined at their corresponding retention times. A reversed phase XTerra MS column (2.1 × 50 mm, 3.5 μm C18; Waters Corpora-
tion, Milford, MA) was used at a flow rate of 200 μl/min with a linear gradient using a mobile phase A consisting of 10 mM triethylamine at pH 11 and a mobile phase B consisting of acetonitrile:methanol (65:35 v/v) containing 10 mM triethylamine. The gradient started at 30% B for initial conditions and increased to 80% B in 5 min. Units of protein activity were defined as 10 times the ratio of milli absorbance unit LTA₄/milli absorbance unit 15-oxy-ETE after the subtraction of the same ratio measured in buffer in the absence of protein. One compli-
cating factor in these analyses was the lipophilicity of LTA₄. Approxi-
mately 5% of the LTA₄ added to the protein or buffer solutions adhered to the wall of the polypropylene tube (data not shown); this added to the variability of the measurements of stabilizing units.

Protein Identification—In-gel tryptic digests were performed using a method previously described (27, 28). Resulting peptide samples were analyzed by electrospray mass spectrometry after MALDI-TOF and MALDI Q-tof-TOF (at 500 ng) was added to 250 μl of buffer or protein solution and incubated at 4 °C. Aliquots (50 μl) were removed at various time points between 2 and 30 min and added to ethanol (100 μl) containing 100 ng of internal standard, which was either 15-oxo-ETE or 20-trifluoro LTA₄. These samples were analyzed using either an in-line photo diode array or a triple quadrupole mass spectrometer (API-3000; PE-Sciex, Thornhill, Canada) as previously described (26). In either case, the log ratio (peak area of LTA₄/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 the stabilization capacity (units of protein activity) was used to follow protein purification. For these assays, LTA₄ (100 ng) was added to either buffer or protein fraction (50 μl). Each sample was allowed to incubate at 4 °C for 20 min, and then ethanolic (100 μl) containing 15-oxy-ETE (100 ng) was added. The sample was brought to initial HPLC conditions by the addition of 150 μl of 10 μM triethyl-
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cating factor in these analyses was the lipophilicity of LTA₄. Approxi-
mately 5% of the LTA₄ added to the protein or buffer solutions adhered to the wall of the polypropylene tube (data not shown); this added to the variability of the measurements of stabilizing units.

RESULTS

Initial studies were designed to critically examine the hy-
pothesis that cells involved in RBL syntheses contain proteins that stabilize this leukotriene and protect it from spontaneous hydrolysis. Cytosol obtained from RBL-1 cells was incubated with LTA₄, and the chemical half-life of LTA₄ in this prepara-
tion was compared with the chemical half-life in buffer. The experiments were performed at 4 °C because the half-life of LTA₄ had been previously shown to be substantially longer at lower temperatures (17). As shown in Fig. 1A, RBL-1 cytosol was able to increase the half-life of LTA₄ to 7.5 ± 0.67 min, a

4.3-fold increase over lysis buffer alone (t½ = 1.4 ± 0.27 min).
Standardized units of stabilizing activity (see “Experimental Procedures”) were used to monitor the protein preparations through subsequent purification steps. Ammonium sulfate precipitation of the cytosolic proteins demonstrated that the activity precipitated between 30 and 75% salt saturation. The number of total activity units found in cytosol decreased when the protein was subjected size exclusion chromatography as shown in Fig. 1 (B and C). However, the specific activity at this stage increased 3.6-fold following size exclusion chromatography. The active fraction eluted from the size exclusion column at a volume corresponding to a molecular mass of 10–25 kDa (Fig. 1B). Without delipidation, the active cytosolic component eluted in the flow through from both cation and anion exchange columns (Fig. 1C and Table I) with up to a 23-fold increase in specific activity. When the protein preparations were delipidated prior to cation exchange, the active component bound to the column and eluted with 160–200 mM sodium chloride (Table I) with essentially the same specific activity.

The molecular mass of the stabilizing protein together with its physicochemical behavior on ion exchange and behavior following delipidation were consistent with a known family of low molecular mass proteins known as the fatty acid-binding proteins (FABPs). To test whether FABPs accounted for the activity found in the anion exchange flow through, this fraction was subjected to an immunoprecipitation, and the supernatant from the immunoprecipitation was tested for any LTA4 stabilizing activity (Fig. 2). Treatment of the anion exchange flow through with the antibody to E-FABP, followed by precipitation of the antibody complex with protein A-Sepharose, completely abolished the stabilizing activity that was previously found in the anion exchange flow through; the units per milliliter decreased from 56 ± 5 to 1.9 ± 2.5 units/ml. When the partially
purified proteins present in the anion exchange flow through fraction were incubated with either nonspecific rabbit IgG or with an antibody to the A-FABP (21), the stabilizing activity remained in the supernatant following the precipitation.

The pelleted protein A beads from the immunoprecipitation were boiled with SDS loading buffer and separated by SDS-PAGE gel electrophoresis. This gel was stained (Fig. 3A) and regions covering the entire gel were analyzed by tryptic digestion followed by MALDI-TOF to determine those proteins bound to the E-FABP antibody. The tryptic peptides obtained from bands at 25 and 50 kDa (bands I and II) in lane 2 of the gel (Fig. 3A) were found to contain peptides derived from rabbit IgG. The only protein identified from the gel, other than rabbit antibody and low levels of keratin, was E-FABP (Band III). The MALDI-TOF mass spectrum of band III in Fig. 3A is shown in Fig. 3B. All peaks marked with asterisks in Fig. 3B were found to match peptides from E-FABP with an average error of 4 ppm. These peptides provided 69% sequence coverage of the protein. Other ions in the mass spectrum result from the autolytic trypsin fragments (m/z 842.49, 1045.57, and 2211.10, marked with T) and tryptic fragments of keratin (m/z 1307.67, 1475.76, and 1791.76).

A separate in-gel tryptic digest of the same gel region was analyzed by electrospray ionization LC/MS/MS, and the resultant spectrum of the HPLC that corresponded to the most abundant MALDI ion at m/z 927.56 was observed as a doubly charged ion (m/z 464.3). Collisional activation of this ion generated a family of product ions consistent with specific peptide cleavages corresponding to y1–y7 for a nonapetide (Fig. 3C). The most abundant product ions were observed at m/z 529.4 (y5) and m/z 359.2 (y3) (Fig. 3C and Table II). The remaining fragment ion in the y ion series was not seen when the doubly charged peptide was collisionally activated, but this y5 ion (m/z 798.5) was seen in the MS/MS spectrum of the singly charged peptide (data not shown) consistent for the tryptic fragment ELGVGLALR. The other major ions seen in the MALDI spectrum (Fig. 3B) were also observed by LC/MS/MS, and the abundant fragment ions from two of these peptides are listed in Table II.

A semi-quantitative Western blot was used to estimate the amount of E-FABP present in RBL-1 cytosol (Fig. 4). Based upon densitometry, the cytosol contained ~0.4 ng of E-FABP/μg of total protein corresponding to ~1–3 pmol/10^6 RBL-1 cells.

For stabilization of LTA_4 by E-FABP, the stabilizing properties of purified delipidated E-FABP were examined using LTA_4 stability units and the half-life of LTA_4. Using a constant concentration of LTA_4 (6 μM), E-FABP reached its maximum of 380 ± 15 units/ml at ~40 μM E-FABP (Fig. 5A). The half-life of LTA_4 (1.5–25 μM) in the presence of 9 μM E-FABP reached a maximum half-life of 29 ± 0.7 min with 3 μM LTA_4, but higher concentrations of LTA_4 had substantially shorter half-lives (Fig. 5B).

Separate studies compared the ability of E-FABP to stabilize LTA_4 relative to bovine serum albumin at both 4 and 37 °C (Fig. 6). The half-life of LTA_4 in buffer alone at 4 °C was 0.6 ± 0.01 min; at 37 °C, the half-life of LTA_4 in buffer was too short to measure. As a negative protein control, ovalbumin was found to have no significant stabilizing activity (data not shown). When bovine serum albumin (10 μM) was tested for its ability to stabilize LTA_4 at 4 °C, LTA_4 had a half-life of 24 ± 1.5 min, and E-FABP (10 μM) stabilized LTA_4 to a half-life of 22 ± 2.5 min. At 37 °C, E-FABP stabilized LTA_4 to a half-life of 7 ± 0.7 min, whereas bovine serum albumin increased the half-life of LTA_4 to 5 ± 0.3 min.

**DISCUSSION**

Fatty acid-binding proteins are a family of low molecular mass proteins (~15 kDa) found in the cytosol of most cells (30). The members of this family have between 20 and 70% sequence identity; however, they all share similar tertiary structures consisting of 10 antiparallel β-strands linked by hydrogen bonds to form a β-barrel (31). The fatty acid-binding protein family has been studied for its involvement in transport of long chain fatty acids as well as various hydrophobic ligands in a number of different tissues (32). This protein family has been implicated as a soluble carrier of insoluble lipids found in the cytoplasm. FabPs also have been proposed to facilitate transport of lipids into cells by sequestration of these hydrophobic

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**Table I**

| Purification step            | Total units | Specific activitya (units/mg) |
|------------------------------|-------------|-----------------------------|
| Cytosol                      | 3080 ± 1100 | 41 ± 10                     |
| Size exclusion pool          | 600 ± 120   | 150 ± 27                    |
| Cation exchange prior to delipidation |            |                             |
| Flow through                 | 360 ± 81    | 950 ± 330                   |
| 160 mM NaCl elution          | NDb         | ND                          |
| Cation exchange following delipidation |            |                             |
| Flow through                 | ND          | ND                          |
| 160 mM NaCl elution          | 220 ± 80    | 700 ± 190                   |

a Specific activity units correspond to an assessment of LTA_4 remaining after 20 min in incubated buffer, normalized to the protein content as measured by the bicinchoninic acid assay.

b ND, not detected.
molecules in the cytosol. Even though the binding properties of these proteins with various ligands have been extensively examined in vitro, there is still some controversy as to their exact physiological role in vivo (33).

Initial studies with crude RBL-1 cell cytosol suggested the presence of a factor that was able to stabilize LTA₄ as measured by an increased half-life of the leukotriene in the cytosol, compared with buffer alone (Fig. 1A). The specific activity of this stabilization was increased by greater than 10-fold using a combination of ammonium sulfate precipitation, size exclusion, and anion chromatography and almost 20-fold using size exclusion chromatography in combination with cation exchange chromatography (Fig. 1B and Table I). The physical and molecular properties of this factor including its molecular mass (10–25 kDa) and retention upon cation exchange chromatography after delipidation suggested that it might be a member of the FABP family. Immunoprecipitation of partially purified RBL-1 cytosol (Fig. 2) with antibodies to E-FABP removed all of the LTA₄ stabilization activity in this fraction. In contrast, when antibodies to A-FABP were utilized, there was little stabilization of LTA₄. E-FABP and A-FABP exhibit 50% amino acid identity and have very similar fatty acid binding

![Image]

**Fig. 3. Identification of the protein immunoprecipitated with anti-E-FABP antibodies.** A, SDS-PAGE of proteins in the pellet following immunoprecipitation of the anion flow through fraction. The brackets indicate the portions of the gel that were subjected to in-gel digestion, and the roman numerals indicate where proteins were identified. B, MALDI-TOF spectrum of the tryptic digest of protein band III from A. The asterisks denote peptides that correspond to peptides predicted from the sequence of E-FABP, and T denotes tryptic autolysis products. C, Electrospray ionization-LC/MS/MS spectrum of the most abundant E-FABP tryptic peptide [M + 2H]⁺ from the MALDI spectrum above (indicated by arrow in B); the sequence of the peptide corresponding to this ion is noted, and the ions matching common peptide fragments from this ion are noted on the spectrum.

| MALDI m/z  | ESI ion collisionally activated | Most abundant product ions (peptide fragment) |
|-----------|-------------------------------|---------------------------------------------|
| 927.55    | 464.4 (±2)                    | 529.4 (y₂); 359.2 (y₂); 381.0 (b₂-H₂O); 551.6 (b₂-H₂O) |
| 1454.66   | 1454.7 (±1)                   | 1014.5 (b₉); 1177.5 (b₁₀); 1308.6 (b₁₁); 1436.7 ([M + H]⁺ – H₂O) |
| 1934.92   | 968.3 (±2)                    | 880.5 (y₇); 1096.5 (y₈); 752.9 (y₆); 853.3 (b₇) |

* Ions correspond to the peptides seen in Fig. 3B.

![Image]

**Fig. 4. Western blot of various concentrations of RBL-1 cytosol (lanes A–C) using anti-E-FABP antibodies.** Lanes D–H correspond to known quantities of recombinant E-FABP. Lane A, 3 μg of RBL cytosol; lane B, 7 μg of RBL cytosol; lane C, 15 μg of RBL cytosol; lane D, 2 ng of E-FABP; lane E, 5 ng of E-FABP; lane F, 10 ng of E-FABP; lane G, 20 ng of E-FABP; lane H, 50 ng of E-FABP.

molecules in the cytosol. Even though the binding properties of these proteins with various ligands have been extensively examined in vitro, there is still some controversy as to their exact physiological role in vivo (33).

Initial studies with crude RBL-1 cell cytosol suggested the
properties, which would suggest that either E-FABP was uniquely active in LTA₄ stabilization or more likely that little if any A-FABP is expressed in RBL-1 cells. Although the antibody to A-FABP cross-reacts with E-FABP protein only slightly (2%), the antibody to E-FABP does not show any measurable cross-reactivity with any other member of the FABP family. Analysis of the antibody-precipitated proteins removed from the purified fraction by the E-FABP antibody led to the identification of rat E-FABP based upon MS analysis of several cross-reactive proteins. The purified fraction by the E-FABP antibody led to the identification of rat E-FABP based upon MS analysis of several cross-reactive proteins. The half-life of LTA₄ in phosphate-buffered saline at 37 °C was too short to measure.

Epithelial FABP (also called keratinocyte FABP) was first identified in murine skin carcinomas and is normally expressed to high levels in epithelial cells of the skin, tongue, lens, lung, and retina and to lesser levels in adipocytes, macrophages, and mammary tissue. Ligand binding studies have revealed high affinity for oleate and arachidonate but little or no affinity for other FABPs. Recent investigations have shown that epoxyeicosatrienoic acids, products of cytochrome P-450 metabolism of arachidonic acid, have also been found to be ligands for other FABPs. Recent investigations have shown that epoxyeicosatrienoic acids can be bound to heart FABP and protect epoxyeicosatrienoic acid from hydrolysis when soluble epoxide hydrolase is added to the buffer (38). Thus, there is a growing body of evidence to suggest that the fatty acid-binding proteins play an important role in eicosanoid biosynthesis and metabolism.

In summary, after purification and immunoprecipitation, a cytosolic protein present in the RBL-1 cell that increased the half-life of LTA₄ from less than 3 s in buffer at 37 °C to greater than 7 min was unambiguously identified as the rat keratinocyte lipid-binding protein. Although the role of E-FABP in leukotriene biosynthesis has not been previously recognized, it is clear that it can play a central role in facilitating distribution of the reactive chemical intermediate, LTA₄, into cellular compartments where subsequent chemical transformations take place to yield the biologically active leukotrienes, LTC₄ and LTD₄. It is also possible that such fatty acid-binding proteins are also critically important in the process.
termed transcellular biosynthesis where LTA₄ is transferred from one cell to another.

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REFERENCES
1. Yokomizo, T., Iizumi, T., Chang, K., Takuwa, Y., and Shimizu, T. (1997) Nature 387, 620–624
2. Evans, J. F. (2002) Prostaglandins Other Lipid Mediat. 68–69, 587–597
3. Shimizu, T., Radmark, O., and Samuelsson, B. (1984) Proc. Natl. Acad. Sci. U. S. A. 81, 689–693
4. Ford-Hutchinson, A. W., Gresser, M., and Young, R. N. (1994) Annu. Rev. Biochem. 63, 383–417
5. Haeggstrom, J. Z. (2000) Am. J. Respir. Crit. Care Med. 161, S25–S31
6. Penrose, J. F., Spector, J., Baldaaro, M., Xu, K., Boyce, J., Arm, J. P., Austen, K. F., and Lam, B. K. (1996) J. Biol. Chem. 271, 11356–11361
7. Fitzpatrick, F. A., Morton, D. R., and Wynalda, M. A. (1982) J. Biol. Chem. 257, 4680–4683
8. Borglat, P., and Samuelsson, B. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 3213–3217
9. Rouzer, C., and Kargman, S. (1988) J. Biol. Chem. 263, 10980–10988
10. Brock, T. G., Payne, R., III, and Peters-Golden, M. (1994) J. Biol. Chem. 269, 22059–22066
11. Dixon, R. A. F., Diehl, R. E., Opas, E., Rands, E., Vickers, P. J., Evans, J. F., Gillard, J. W., and Miller, D. K. (1990) Nature 343, 282–284
12. Leslie, C. C. (1997) J. Biol. Chem. 272, 16709–16712
13. Sala, A., Bolla, M., Zarini, S., Muller-Peddinghaus, R., and Folco, G. (1996) J. Biol. Chem. 271, 17944–17948
14. McGee, J., and Fitzpatrick, F. A. (1986) Proc. Natl. Acad. Sci. U. S. A. 83, 1349–1353
15. Feinman, S. J., and Cannon, P. J. (1986) J. Biol. Chem. 261, 16466–16472
16. Macleod, J. A., and Murphy, R. C. (1988) J. Biol. Chem. 263, 174–181
17. Peters, P., Jr. (1996) All About Albumin, pp. 76–132, Academic Press, New York
18. Goetze, A. M., Fayer, L., Bouska, L., Bornemeier, D., and Carter, G. W. (1985) Prostaglandins 29, 689–701
19. Jakschik, B. A., and Kao, C. G. (1983) Prostaglandins 25, 767–782
20. Carrier, D. J., Bogrii, T., Cosentino, G. P., Guse, I., Rahbit, S., and Singh, K. (1988) Prostaglandins Leukot. Essent. Fatty Acids 34, 27–30
21. Hertzel, A. V., Bennars-Eiden, A., and Berndhohr, D. A. (2002) J. Lipid Res. 43, 2105–2111
22. Zarini, S., and Murphy, R. C. (2003) J. Biol. Chem. 278, 11190–11196
23. England, S., and Seifler, S. (1990) in Guide to Protein Purification (Deutscher, M. P., ed) pp. 285–297, Academic Press, San Diego, CA
24. Glanz, J. F. C., and Veerkamp, J. H. (1983) Anal. Biochem. 132, 89–95
25. Laemmli, U. K. (1970) Nature 227, 680–685
26. Dickinson, J. F. C., and Veerkamp, J. H. (1992) Anal. Biochem. 203, 173–179
27. Kauffman, L., Wernstedt, C., Gonez, J., and Henson, P. M. (1995) Anal. Biochem. 224, 451–455
28. Perkins, D. N., Pappin, D. J. C., Creasy, D. M., and Cottrell, J. S. (1999) Electrophoresis 20, 3551–3567
29. Storch, J., and Thoumer, A. E. A. (2000) Biochem. Biophys. Acta 1486, 28–44
30. Sacchettini, J. C., Gordon, J. I., and Banaszk, L. J. (1989) J. Mol. Biol. 208, 327–339
31. Watersman, A. W., van Meerk, H. T. B., and Veerkamp, J. H. (2001) Int. J. Biochem. Cell Biol. 33, 865–876
32. Krieg, P., Feil, S., Forstenberger, G., and Bovden, G. T. (1993) J. Biol. Chem. 268, 17862–17869
33. Bonnaerts-Eiden, A., Haggstrom, J. Z., and Banaszk, A. M. (1996) Biochemistry 35, 2894–2900
34. Gutierrez-Gonzalez, L. H., Ludwig, C., Hohoff, C., Rademacher, M., Hanhoff, T., Beterjans, H., Spener, F., and Lucke, C. (2002) Biochem. J. 364, 725–737
35. Hohoff, C., Borchers, T., Rustow, B., Spener, F., and Van Tilbeurgh, H. (1999) Biochemistry 38, 12229–12339
36. Zimmerman, A. W., van Meerk, H. T. B., and Veerkamp, J. H. (2001) Int. J. Biochem. Cell Biol. 33, 865–876
37. Carrier, D. J., Bogrii, T., Cosentino, G. P., Guse, I., Rahbit, S., and Singh, K. (1988) Prostaglandins Leukot. Essent. Fatty Acids 34, 27–30
38. Widstrom, R. L., Norris, A. W., Van Der Veer, J., and Spector, A. A. (2003) Biochemistry 42, 11762–11767