Irreversible Inactivation of 5-Lipoxygenase by Leukotriene A₄

CHARACTERIZATION OF PRODUCT INACTIVATION WITH PURIFIED ENZYME AND INTACT LEUKOCYTES*

(Received for publication, July 27, 1993, and in revised form, September 20, 1993)

Robert A. Lepley‡ and F. A. Fitzpatrick
From the Department of Pharmacology C236, University of Colorado Health Science Center, Denver, Colorado 80262

We report that leukotriene A₄, the electrophilic product of 5-lipoxygenase catalysis, irreversibly inactivates the enzyme. Leukotriene A₄ inhibits 5-hydroxyeicosatetraenoic acid formation by human neutrophils and differentiated granulocytic HL-60 cells in a concentration-dependent manner with IC₅₀ values = 22.4 ± 2.5 and 29.0 ± 8.0 μM, respectively. Recovery of cellular enzymatic activity is negligible (<8%) following inactivation. Leukotriene A₄ inactivates cellular 5-lipoxygenase without inhibiting its translocation from the cytosol to the membrane, suggesting that it impairs catalysis without impairing formation of the complex between 5-lipoxygenase and its membrane-associated activating protein. Consistent with this, leukotriene A₄ inactivates purified 5-lipoxygenase from human neutrophils, via saturable, pseudo first-order kinetics with a rate constant, kᵢ = 0.14 min⁻¹ and a dissociation constant, Kᵢ = 2.1 ± 0.7 μM. Purified 5-lipoxygenase incubated with [³H]arachidonic acid incorporated a radiolabeled species that was not removed by electrophoresis under reduced denaturing conditions. Preincubation with leukotriene A₄ diminished the incorporation of radiolabeled material, consistent with irreversible modification of 5-lipoxygenase by its metabolite product, leukotriene A₄. This unusual product inactivation mechanism may contribute to the decline in 5-lipoxygenase activity observed during catalysis.

We hypothesized that LTA₄, an electrophilic epoxide, is also capable of reacting with certain other macromolecules. LTA₄ is the product of 5-LO/5-LO catalysis; therefore it could exist proximal to the enzyme active site at some stage in the catalytic cycle. Thus, we investigated the inactivation of 5-LO by LTA₄. The results with intact neutrophils, HL-60 cells, and with isolated enzyme suggest that LTA₄ participates directly in the inactivation process which accompanies 5-LO catalysis.

EXPERIMENTAL PROCEDURES

Materials—LTA₄, methyl ester, LTB₄, 5(S)-HETE, 12(S)-HETE, 18(S)-HETE, 9(S)-hydroxyoctadecadienoic acid, and prostaglandin B₁ (Cayman Chemical); arachidonic acid and linoleic acid (NuChek Prep); ATP-Sepharose resin, l-α-phosphatidylycholine (Sigma); PD-10 gel filtration columns (Pharmacia LKB Biotechnology Inc.); MK-886 (3-[1-(4-chlorobenzyl)]-3-butyryl-thio-isopropyl-indol-2-yl)-2,2-dimethylpropionic acid) (13) and anti-5-LT antisemur (LO-32) were supplied by Dr. Jillian Evans (Merck Frosst) (14, 15); zileuton, N-[1-benzo-thien-2-yl)-N'-hydroxyurea; goat anti-rabbit antisemur-conjugated to horseradish peroxidase (Bio-Rad) and ECL enhanced chemiluminescence reagents (Amer sham) were used. LTA₄ methyl ester was saponified in acetone with LiOH (16). Human neutrophils were isolated from whole blood by sedimentation through dextran followed by density gradient centrifugation (17). HL-60 cells (American Type Culture Collection) were differentiated into granulocyte-like cells by incubation with 1.35% dimethyl sulfoxide (v/v) in RPMI 1640 medium supplemented with 20% heat-inactivated fetal calf serum, 2 μM 1-glutamine and penicillin/streptomycin. 5-LO activity in HL-60 cells was maximal by 6 days, consistent with previous observations (18).

Inactivation of Cellular 5-LO by LTA₄—Human neutrophils (1.0 ml, 2 x 10⁶ cells/ml) or differentiated HL-60 cells, both prominent sources of 5-LO and FLAP (14, 15, 19–21), were incubated with 0–100 μM LTA₄ for 5 min at 37 °C. An aliquot (50 μl) was removed for quantitation of LTB₄ to verify that LTA₄ reached the cytosol and to verify that no 5-HETE was formed during this incubation. The cells were then stimulated with 5 μM Ca²⁺-ionophore A23187 for 3 min to activate 5-LO, reflected by 5-HETE formation. The reaction was quenched by acidification to pH 1; samples (1.0 ml) were diluted with 1 ml of 0.9% (w/v) NaCl; extracted with ethyl acetate/hexane (3 x 2 ml, 1/1, v/v); the organic phases were pooled, dried under N₂, and reconstituted in 200 μl of ethanol/H₂O, 1/1 (v/v), containing 1 nmol of prostaglandin B₁ as an internal standard for quantitation. Sample components were eluted from a C₁₈ column (Beckman Ultrasphere ODS, 250 x 4-mm inner diameter) using a gradient of 72.5% solvent B (60/40 (v/v) acetonitrile/methanol), 37.5% solvent A (water) to elute prostaglandin B₁, 5,12-diHETEs, and LTB₄, then increasing to 80% solvent B after 18 min to elute 5(S)-HETE. The UV detector was operated at 280 nm to monitor the elution of prostaglandin B₁ at 11–12 min, nonenzymatic 5,12-diHETEs at 13 min, and LTB₄ at 14 min; the detector was operated at 234 nm to monitor the elution of 5(S)-HETE at 27 min. Quantitation was based on integrated peak areas calibrated with standards of known concentration. In corresponding experiments we determined if LTA₄ methyl ester, LTB₄, and nonenzymatic 5,12-diHETEs inhibited cellular 5-LO activity in neutrophils and HL-60 cells.

Immunochromatographic Determination of 5-LO Translocation from Cytosol to Membrane—Control cells or HL-60 cells (1 ml, 2 x 10⁶ cells/ml) were incubated with either 50 μM LTA₄ or 0.90 μM MK-886 for 5 min at 37 °C, then stimulated with 5 μM A23187 for 3 min. Samples (6–10 ml) were quenched in EGTA, 5 mm final concentration, then centrifuged at 300 x g for 12 min. The cells were resuspended in 0.05 M phosphate, pH 7.1, 0.1 M NaCl, 2 mM EDTA, 0.5 mM phenylmethylsulfonyl fluoride, 60
μg/ml soybean trypsin inhibitor, and disrupted by sonication for 3 x 20 s (75% duty cycle, power setting 3). After centrifugation at 100,000 x g for 1 h at 4 °C, 200 μl of the membrane fraction (4-5 μg/ml) and cytosolic fraction (1-2 μg/ml) was immediately mixed with 100 μl of 3 x electrophoresis buffer (20 mM Tris-HCl, pH 6.8, 0.4% (w/v) SDS, 4% glycerol, 0.24 mM β-mercaptoethanol and bromphenol blue), boiled for 5 min, and aliquots were applied to 10% acrylamide gel with 4% stacking gel. After gel electrophoresis, proteins were transferred to nitrocellulose membranes. Membranes were saturated with 5% non-fat dried milk to reduce nonspecific binding, then incubated with L032 anti-5-LO antibody (1:15, 1:400 dilution for 2 h. The membrane was then washed 4 x with TBS (25) and Tween TBS and incubated for 1 h with goat anti-rabbit antiserum. Enzyme from HL-60 cells (1.5-3.5 × 10^6 cells) was applied at a flow rate of 15 ml h^-1. Membranes were washed 4 x after incubation with 0.5 μg/ml of purified 5-HT (30-40 pg/ml); samples (100 μl) were applied to a 10 x 10 cm 5% acrylamide gel. Immediately, the membrane was transferred to a 10% polyacrylamide stacking gel. Radiolabeled protein was detected by autoradiography with Kodak X-AR film exposed for 4 weeks at -70 °C. In corresponding experiments 5-LO was incubated with 5 μg LTA for 3 min, prior to incubation with [3H]arachidonic acid, to determine if this reduced incorporation of radiolabeled metabolites into the enzyme.

RESULTS  
LTAg, a product of 5-LO catalysis, inhibited 5-HETE formation by human neutrophils in a concentration-dependent manner. Half-maximal inhibition (IC₅₀) required 22.4 ± 2.5 μg LTA for LTA₄ (Fig. 1, lower panel). Inhibition originated from exogenously added LTA₄, not from LTB₄ generated by the neutrophils. First, LTB₄ formed from LTA₄ was always <0.5 μM in these experiments. However, LTB₄, even at a 10-fold higher concentration, had no significant effect on 5-HETE formation. For instance, neutrophils incubated with 1, 3, or 6 μg LTB₄ formed 1.26 ± 0.13, 1.40 ± 0.17, and 0.88 ± 0.25 nmol of 5-HETE/2 x 10⁷ cells, respectively. These values (mean ± S.E., n = 4) were statistically indistinguishable (p > 0.05) from the control value of 1.53 ± 0.18 nmol of 5-HETE/2 x 10⁷ cells (mean ± S.E., n = 5). Second, LTA₄ methyl ester, which is not converted into LTB₄ or LTB₃ methyl ester, also inhibited 5-HETE formation with a...
similar potency: $IC_{50} = 27.0 \pm 1.8$ µm (Fig. 1, lower panel). Results were equivalent with HL-60 cells, a granulocyte-like cell line containing 5-LO and FLAP, but distinct from neutrophils in other respects. LTA₄ and LTA₄ methyl ester each inhibited 5-HETE formation in a concentration-dependent manner with $IC_{50} = 29.0 \pm 8.0$ and $19.9 \pm 5.2$ µm, respectively (Fig. 1, upper panel). LTA₄ acted irreversibly: HL-60 cells incubated with 50 µm LTA₄, then washed twice with Ca²⁺-free buffer prior to stimulation with 5 µm A23187, formed only 0.02 nmol of 5-HETE/10⁷ cells, a negligible amount (<6%) compared to the control value of 0.32 nmol of 5-HETE/10⁷ cells in this particular experiment. Results were similar for LTA₄ methyl ester.

5-LO enzyme occurs predominantly in the cytosol of HL-60 cells under basal conditions (Fig. 2, lane 1). Stimulation with A23187 promotes its translocation from the cytosol to the cell membrane, consistent with results by others (15) (Fig. 2, lane 2). We verified that this process was blocked by MK-886, a novel leukotriene biosynthesis inhibitor which impairs the translocation of 5-LO and its subsequent interaction with FLAP (Fig. 2, lane 3). In contrast, LTA₄ at concentrations which inhibited 5-HETE formation >90% did not inhibit the translocation of 5-LO from cytosol to membranes (Fig. 2, lane 4). The ratio of cytosolic 5-LO/membrane-associated 5-LO in cells incubated with 50 µm LTA₄, prior to stimulation with A23187 (lane 4), was indistinguishable from corresponding control cells (lane 2). This implies that LTA₄ inactivated 5-LO in the cytosol prior to its translocation, and that its inactivation does not impair formation of the FLAP-5-LO complex (27).

Experiments with purified enzyme isolated from human leukocytes confirmed that LTA₄ inactivated 5-LO in the absence of FLAP. Activity declined exponentially as a function of time after addition of LTA₄ (Fig. 3, upper panel). For three separate enzyme preparations, half-maximal loss of activity ($t_{1/2}$) occurred 10.7 ± 2.0 s after addition of 2 µm LTA₄. The time for half-inactivation ($t_{1/2}$) plotted as a function of LTA₄ concentration showed that inactivation was saturable, with an apparent rate constant, $k_{inact} = 0.14$ s⁻¹ and a dissociation constant, $K_d = 1.1$ µm LTA₄ (Fig. 3, upper inset). Results were similar for LTA₄ methyl ester: 5-LO activity declined exponentially with half-maximal loss of activity at 8.9 s after addition of 10 µm LTA₄ methyl ester. Inactivation was saturable with an apparent rate constant, $k_{inact} = 0.08$ s⁻¹ and a dissociation constant, $K_d = 5.7$ µm (Fig. 3, lower panel and inset). Dixon plots (µm inhibitor versus $1/V_{initial}$) were linear and yielded similar estimates for the dissociation constants of LTA₄ and LTA₄ methyl ester (Table I).

![Diagram of enzyme activity](image)

**Figure 2.** Effect of LTA₄ on translocation of 5-LO in HL-60 cells. HL-60 cells were incubated with 0.2 µm MK-886 or 50 µm LTA₄, then stimulated with 5 µm A23187 to initiate translocation of 5-LO from cytosol to membranes. 5-LO in the 100,000 x g supernatant (cytosol) and the 100,000 x g pellet (membranes) was monitored immunochemically with a 5-LO specific antiserum. The amount of membrane-associated enzyme is depicted directly above the amount of cytosolic enzyme. Lane 1, unstimulated cells. Lane 2, cells + A23187. Lane 3, cells + MK-886 + A23187. Lane 4, cells + LTA₄ + A23187.

**Figure 3.** Time-dependent inactivation of purified 5-LO by LTA₄ and LTA₄ methyl ester. Upper panel, LTA₄ inactivates purified 5-LO by LTA₄ (O) via pseudo first-order kinetics. Kitz-Wilson plot ($t_{1/2}$ versus µm⁻¹ LTA₄) Upper panel inset, yield an inhibitor dissociation constant $K_d = 1.1$ µm LTA₄ and an inactivation rate constant, $k_{inact} = 0.14$ s⁻¹. Lower panel, pseudo-first-order inactivation kinetics and Kitz-Wilson plot for LTA₄ methyl ester.

**Table I**

| 5-LO preparation | Calculations | $K_d$ (µm LTA₄) | $k_{inact}$ (s⁻¹) |
|------------------|--------------|----------------|------------------|
| 1                | Dixon plot   | 1.3            |                  |
| 2                | Dixon plot   | 1.4            |                  |
| 3                | Kitz-Wilson plot | 1.1 | 0.14 s⁻¹ |
| 4                | Kitz-Wilson plot | 4.3 | 0.18 s⁻¹ |
| 5                | Dixon plot   | 10.3           | 0.084 s⁻¹ |

Gel filtration of the LTA₄-5-LO complex did not restore enzymatic activity, consistent with irreversible binding of LTA₄ to 5-LO. Enzyme incubated with 5 µm LTA₄ for 3 min at 25°C, then gel-filtered through PD-10 columns, had a specific activity of $1.1 \times 10^{-2}$ nmol of 5-HETE µg⁻¹ min⁻¹ after gel filtration. This was a 54% reduction compared to a corresponding control: $2.4 \times 10^{-2}$ nmol of 5-HETE µg⁻¹ min⁻¹. Enzyme incubated with 50 µm LTA₄ for 3 min, and then gel-filtered, had a specific activity of 0.78 $\times 10^{-2}$ nmol of 5-HETE µg⁻¹ min⁻¹, a 68% reduction compared to the control. Gel electrophoresis indicated that 5-LO was radio labeled during catalysis of [³H]arachidonic acid (Fig. 4) and that preincubation of 5-LO with LTA₄ prior to incubation with [³H]arachidonic acid reduced the in-
corporation of radiolabel. Irreversible inactivation by LTA, did not alter the sequestration of 5-LO to an ATP affinity column. Control and inactivated 5-LO each bound to the column and each emerged in fractions 4–6 when the column was eluted with buffer containing 20 mM ATP (data not shown). Zileuton, a reversible inhibitor which binds to the active site of 5-LO, did not protect it from inactivation by LTA, (Table II).

LTA₄ also inhibited 5-LO isolated from potatoes. Enzyme preincubated with 25 μM LTA₄ converted linoleic acid into 0.30 ± 0.06 nmol of 9(S)-hydroxyoctadecadienoic acid/ml, a value 71% less than the control, 1.04 ± 0.12 nmol/ml. Results were similar with 25 μM LTA₄ methyl ester: conversion of linoleic acid declined by 72%. Potato 5-LO, like 5-LO isolated from human neutrophils, does not require FLAP for activity in vitro (1, 26). We also determined the effect of LTA₄ on human platelet 12-LO, a lipoxygenase with different regiospecificity and with no requirement for cellular translocation. LTA₄ inhibited 12-LO in a concentration-dependent manner with an IC₅₀ = 72 μM. 12-HETE formation declined from the control value, 18.4 ± 0.7, and 6.7 ± 0.3 nmol/ml in the presence of 15, 25, 50, and 75 μM LTA₄, respectively.

**DISCUSSION**

Inactivation of lipoygenase activity has been investigated periodically since its discovery by Smith and Lands (7). With 15-LO from plants (7, 28, 29) or reticulocytes (8–10) the decline in enzyme activity accompanying catalysis is rapid under aerobic conditions. The exact cause of the suicide process is uncertain; however, it is usually attributed to radical species derived from homolytic cleavage of the hydroperoxy lipid by a peroxidase activity intrinsic to lipoygenase enzymes. In support of this hypothesis, exogenously added lipid hydroperoxides, identical to those generated during catalysis, will inactivate 15-LO by a pseudo first-order process (29), and radical scavengers will protect the enzyme from suicide inactivation. It should be stressed that radical-mediated inactivation is one likely mechanism, but not the only one. Catalysis of polyunsaturated fatty acids by mammalian and soybean 15-LO generates chemically reactive products, including allylic epoxides and α,β-unsaturated ketones (30–33) which could also contribute to inactivation by covalently modifying the enzyme.

Mammalian 5-LO also undergoes suicide inactivation but there is less information available about this enzyme (3), and precedents from 15-LO or vegetable 5-LO (34) may have limited value, considering the differences in the enzymology of the proteins. For example, the isolated mammalian 5-LO requires ATP and Ca²⁺ for catalysis; it has two regiospecific lipoygenase activities, 5-LO and 8-LO; and in cells its translocation from the cytosol to the membrane is necessary for expression of maximal activity. 5-LO generates two products, 5(S)-HPETE and LTA₄, which could each contribute to irreversible inactivation. The former could act via a radical mediated process, the latter via covalent attachment to susceptible nucleophiles essential for lipoygenase activity. Aharony et al. (3), using crude cytosolic preparations, have established that 5(S)-HPETE can inactivate 5-LO. Using enzyme purified to near homogeneity, we conclude that LTA₄ is also capable of inactivating 5-LO via a saturable, time-dependent, first-order process. Our results are compatible with those of Aharony et al. (3) who acknowledged the possibility that additional intermediates in the reaction pathway from the enzyme-5-HPETE complex (E-S) to the enzyme-product complex (E-P) could be part of the inactivating mechanism. Aharony et al. (3) attributed a thiol-mediated protection of 5-LO to the destruction of 5-HPETE, but it is equally plausible that thiol protects a nucleophile on the enzyme from reaction with LTA₄. LTA₄ inactivates isolated 5-LO 10 times faster than 5-HPETE, while 5-HPETE is more potent (3), suggesting that there are two discrete reactions involved.

Two types of data suggest that LTA₄ acts via covalent attachment to 5-LO. These include: (i) detection of 5-LO labeled during catalysis of radioactive substrate and the stability of this labeled species during electrophoresis under reduced, denaturing conditions; and (ii) irreversibility of the LTA₄-5-LO complex by gel-filtration chromatography. There are precedents showing that eicosanoids can covalently modify the enzymes participating in their biosynthesis. LTA₄ hydrolyase is the most thoroughly characterized example (12, 35–37). Other examples, based on detection of enzyme labeled irreversibly during catalysis of radioactive substrate are platelet cyclooxygenase (38) and thromboxane synthase (39). Our results show that LTA₄ can be included in this category. The chemical nature of the modification is uncertain but the available data are consistent with nucleophilic attack by LTA₄. At present, using the reliable, one-step affinity purification procedure we have been unable to obtain enough enzyme of suitable purity (99%) for electrospray mass spectrometric characterization of the modified enzyme. This would be useful to establish the stoichiometry of the reaction.

It is notable that LTA₄ inactivates 5-LO in leukocytes. Its lower apparent potency in cells, compared to purified enzyme, probably originates from more complex metabolism and disposition. In neutrophils and HL-60 cells exogenously supplied LTA₄ decays rapidly via both enzymatic and nonenzymatic hydration, thus the concentration which crosses the membranes, reaches the cytosol, and encounters 5-LO is proportional to, but
less than the amount of LTA added. In contrast to a recent report (40) showing a partial inhibition of LTB₄ formation by LTA₄, we found a complete, dose-dependent inhibition. Furthermore, we found no detectable effect of LTA₂ on sulfidopeptide formation. The increase in sulfidopeptide formation reported (40) in neutrophils treated sequentially with LTA₄, then A23187, is incompatible with our data showing that LTA₄ inactivates 5-LO. Contamination of the neutrophil preparations in Ref. 40 with platelets could explain some of the discrepancies.

Our data with cells and with purified 5-LO indicate that the enzyme itself is the target for inactivation by LTA. Consistent with this conclusion, LTA₄ did not inhibit 5-LO translocation implying that 5-LO/FLAP interactions can occur with inactive enzyme. However, recent data from Evans and colleagues (27) suggest that the translocation and activation of 5-LO are discrete events. In view of this, we cannot exclude the possibility that LTA₄ reacts with FLAP and impairs the activation step without impairing the formation of the 5-LO-FLAP complex. In summary, our results are consistent with the following scheme for irreversible, product inhibition of 5-lipoxygenase.

\[ K_i \]

\[ \text{LTA}_4 \text{ + 5-LO} \rightarrow [\text{LTA}_4\text{-5-LO}] \rightarrow \text{inactive 5-LO} \]

**Scheme I**

At present, the only two examples of covalent modification of macromolecules by LTA₄ are LTA₄ hydrolyase and 5-LO. It is interesting to speculate that unrestrained LTA₄ formation could generate sufficient material to modify other macromolecules in certain *in situ* situations and this might contribute to the pathophysiology of inflammation.

**Acknowledgment**—Dr. Jillian Evans of Merck-Frost provided anti-5-LO antiserum.

**REFERENCES**

1. Shimizu, T., Radmark, O., and Samuelsson, B. (1984) *Proc. Natl. Acad. Sci. U. S. A.* 81, 689-693
2. Shimizu, T., Isumi, T., Seyanne, Y., Tadokoro, R., Radmark, O., and Samuelsson, B. (1986) *Proc. Natl. Acad. Sci. U. S. A.* 83, 4175-4179
3. Aharonov, D., Redkar-Brown, D. G., Hubbs, S. J., and Stein, R. L. (1987) *Prostaglandins 33,* 85-100
4. Sun, F. F., and McGuire, J. C. (1984) *Biochim. Biophys. Acta 794,* 56-64
5. Wong, A., Hwang, S. M., Cook, M., Hogaaboem, G., and Crooke, S. (1988) *Biochemistry 27,* 6765-6769
6. Goetz, A. M., Fayer, L., Bouska, J., Birnmeier, D., and Carter, G. W. (1985) *Prostaglandins 29,* 689-701
7. Smith, W. L., and Lands, E. M. (1972) *J. Biol. Chem. 247,* 1038-1047
8. Hartel, B., Ludwig, P., Schewe, T., and Rapaport, S. M. (1982) *Eur. J. Biochem. 126,* 355-357
9. Rapaport, S. M., Schewe, T., Weinsen, R., Hulangk, W., Ludwig, P., Janicke-Hohne, Tannert, C., Hiebsch, C., and Klett, D. (1979) *Eur. J. Biochem. 86,* 545-561
10. Rapaport, S., Hartel, B., and Hausdorf, G. (1984) *Eur. J. Biochem. 139,* 573-576
11. Taylor, L., Menconi, M. J., and Polgar, P. (1983) *J. Biol. Chem. 258,* 6855-6857
12. Orning, L., Gierse, J., Duffin, K., Krivi, G., and Fitzpatrick, P. A. (1990) *J. Biol. Chem. 265,* 27373-27379
13. Gallard, J., Ford-Hutchinson, A. W., Chan, C., Charleson, S., Denia, D., Foster, A., Fortin, R., Leger, S., McFarlane, C. S., Morten, H., Pichuta, H., Rien- deau, D., Rouzer, C., Raksh, Y., Young, R., Mackinry, D. E., Peterson, L., Bach, T., Biermann, G., Hopple, S., Humes, J., Hipe, L., Luedl, S., Metzger, J., Meurer, R., Miller, D., Opea, E., and Pacholok, S. (1986) *Can. J. Physiol. Pharmacol. 67,* 465-464
14. Rouzer, C., and Kargman, S. (1988) *J. Biol. Chem. 263,* 10980-10988
15. Kargman, S., Pratil, P., and Evans, J. F. (1991) *J. Biol. Chem. 266,* 23745-23752
16. Carrier, D. J., Boggi, T., Coesentino, G. P., Guei, I., Rakhi, S., and Singh, K. (1998) *Prostaglandins, Leukotrienes Essent. Fatty Acids 54,* 27-30
17. Young, R., Worthing, G. S., Hasket, C., Tunnessen, M. G., and Henson, P. M. (1990) *Am. J. Respir Cell. Mol. Biol. 2,* 523-532
18. Kargman, S., and Rouzer, C. (1989) *J. Biol. Chem. 264,* 13313-13320
19. Samuelsson, B., and Punk, C. D. (1980) *J. Biol. Chem. 265,* 19469-19472
20. Rouzer, C., Ford-Hutchinson, A. W., Morten, H., and Gallard, J. (1990) *J. Biol. Chem. 265,* 1438-1442
21. Charleson, S., Pratil, P., Leger, S., Gallard, J. W., Vickers, P. J., Macinti, J. A., Charleisen, P., Guey, J., Ford-Hutchinson, A. W., and Jones, J. F. (1992) *Mol. Pharmacol. 41,* 873-879
22. Casby, M., Peters-Golden, M., Fantone, J., III, and Sporn, P. (1992) *J. Biol. Chem. 267,* 570-576
23. Wiseman, J. S. (1989) *Biochemistry 28,* 2106-2111
24. Denne, D., Falguyrat, J., Rendeau, D., and Abramowitz, M. (1991) *J. Biol. Chem. 266,* 5072-5079
25. Kitz, R., and Wilson, I. B. (1962) *J. Biol. Chem. 237,* 3245-3250
26. Mulliez, E., Leblanc, J-P., Girerd, J. J., Rigaud, M., and Chottard, J. C. (1984) *Biochim. Biophys. Acta 816,* 13-23
27. Kargman, S., Vickers, P., and Evans, J. F. (1992) *J. Cell. Biol. 119,* 1701-1709
28. Cho, K. S., and Tappel, A. L. (1969) *Biochemistry 8,* 2837-2852
29. Sok, D. E., and Kim, M. R. (1989) *Biochem. Biophys. Res. Commun. 162,* 1357-1362
30. Van Os, C. F. A., Rijke-Schidler, G. P. M., Van Halbeck, H., Verhagen, J., and Vliegenthart, J. F. (1981) *Biochem. Biophys. Acta 683,* 177-183
31. Lundberg, U., Radmark, O., Maismenst, C., and Samuelsson, B. (1981) *FEBS Lett. 126,* 127-132
32. Narusuya, S., Salmon, J., Cottet, F. H., Weatherly, B. C., and Flower, R. J. (1981) *J. Biol. Chem. 236,* 9583-9592
33. Mass, R. L., and Brash, A. R. (1985) *Proc. Natl. Acad. Sci. U. S. A.* 86, 2984-2988
34. Kim, M. R., Kinn, S. H., and Sok, D-R. (1989) *Biochem. Biophys. Res. Commun. 164,* 1384-1390
35. Pratt, S. (1984) *J. Biol. Chem. 260,* 7615-7621
36. Evans, J., Nathaniel, D., Zamboni, R., and Ford-Hutchinson, A. W. (1985) *J. Biol. Chem. 260,* 10966-10970
37. Evans, J., and Kargman, S. (1992) *FEBS Lett. 297,* 129-142
38. LeComte, M., Leocq, R., Dumont, J. E., and Boyns, M. J. (1990) *J. Biol. Chem. 265,* 5178-5187
39. Joves, D., and Fitzpatrick, F. A. (1991) *J. Biol. Chem. 266,* 23510-23514
40. Hilger, R. A., and Kong, W. (1992) *Immunology 77,* 408-415