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Single protein from human leukocytes possesses 5-lipoxygenase and leukotriene A_4 synthase activities

(5-hydroperoxyicosatetraenoic acid/5-hydroxyicosatetraenoic acid/inflammation/enzyme regulation)

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ABSTRACT **The activity of leukotriene A_4 (LTA_4) synthase in crude human leukocyte homogenates was found to have a similar requirement for Ca^{2+} and ATP as had been noted previously for 5-lipoxygenase activity. Purification of the 5-lipoxygenase using ammonium sulfate fractionation, AcA 44 gel-filtration chromatography, and HPLC on anion-exchange and hydroxyapatite columns demonstrated that LTA_4 synthase activity copurified with the 5-lipoxygenase with similar recoveries and increases in specific activity. Furthermore, the two enzymatic activities coeluted exactly on three different HPLC systems. Maximal activity of purified LTA_4 synthase required the addition of three nondialyzable stimulatory factors, two of which were cytosolic and one of which was membrane-bound. These findings were identical for 5-lipoxygenase activity. When incubated with arachidonic acid, the purified 5-lipoxygenase converted ≈15% of its endogenously generated 5-hydroperoxyicosatetraenoic acid (5-HPETE) to LTA_4. LTA_4 production was more efficient when the enzyme utilized 5-HPETE generated from arachidonic acid than when 5-HPETE was exogenously supplied as substrate. These findings suggest that a single protein from human leukocytes possesses 5-lipoxygenase and LTA_4 synthase activities and that the synthesis of LTA_4 from 5-HPETE is controlled by the same complex multicomponent system that regulates the 5-lipoxygenase reaction.**

The leukotrienes (LTs) are a recently characterized class of arachidonic acid metabolites whose potent biological activities suggest an important role in the mediation of inflammation and immediate hypersensitivity (1). A thorough understanding of the biosynthetic regulation of the LTs should, therefore, significantly augment our knowledge of the pathophysiology of these immunologic processes.

The first step in the biosynthesis of LTs is catalyzed by the enzyme 5-lipoxygenase, which peroxidizes arachidonic acid at carbon 5 to form (5*S*)-hydroperoxy-6-*trans*-8,11,14-*cis*-icosatetraenoic acid (5-HPETE). This compound is then converted to the unstable epoxide intermediate, LTA_4 (5,6-epoxy-7,9-*trans*-11,14-*cis*-icosatetraenoic acid) by the enzyme LTA_4 synthase. Depending upon the enzymes available, LTA_4 may be subsequently hydrolyzed to (5*S*),(12*R*)-dihydroxy-6,14-*cis*-8,10-*trans*-icosatetraenoic acid (LTB_4) or it may be converted, by means of addition of glutathione at carbon 6, to LTC_4 [(5*S*)-hydroxy-(6*R*)-glutathionyl-7,9-*trans*-11,14-*cis*-icosatetraenoic acid]. LTA_4 may also undergo nonenzymatic hydrolysis to form stereochemical isomers of LTB_4, which have been designated compounds I and II (2, 3).

Recently, the successful purification of 5-lipoxygenase from human leukocytes and RBL cells has been reported (4, 5). The studies of the 5-lipoxygenase from human leukocytes have resulted in significant progress in the understanding of the regulation of this enzyme (4, 6). The enzyme is an unstable, hydrophobic, 80-kDa protein that requires multiple stimulatory factors for maximal activity. These include Ca^{2+}, ATP, and at least three nondialyzable components, two of which are soluble and one of which is membrane-bound. Thus, this enzyme appears to be controlled by a complex, multicomponent regulatory system (4, 6).

In contrast to the increasing knowledge of the 5-lipoxygenase, very little is known about the LTA_4 synthase. Studies from this laboratory showed that an enzyme from potatoes that possesses 5- and 8-lipoxygenase activities could catalyze the synthesis of LTA_4 from either arachidonic acid or 5-HPETE (7). The nonspecific conversion of 5-HPETE to LTA_4 by hemoproteins has also been reported (8). However, there has not yet been an extensive study of the specific mammalian LTA_4 synthase.

In this paper we report the results of experiments that show that the LTA_4 synthase reaction in human leukocyte homogenates requires Ca^{2+} and ATP in concentrations similar to those required by the 5-lipoxygenase. We further show that LTA_4 synthase activity copurifies with 5-lipoxygenase and that the same cytosolic and membrane-bound stimulatory factors are required for both reactions. These results suggest that, in human leukocytes, a single enzyme is responsible for the production of 5-HPETE from arachidonic acid and for its subsequent conversion to LTA_4.

MATERIALS AND METHODS

Enzyme Purification. 5-Lipoxygenase was purified from homogenates (10,000 × *g* supernatants) of human leukocyte suspensions (500–600 ml at 150–200 × 10^6 cells per ml) by a combination of ammonium sulfate fractionation, AcA 44 gel-filtration chromatography, and HPLC on hydroxyapatite and the anion-exchanger Mono Q. These methods are described elsewhere (4).

Preparation of Stimulatory Factors. Components that stimulate 5-lipoxygenase activity have been isolated during the course of enzyme purification. One of these is membrane-associated and is obtained by the centrifugation of a leukocyte homogenate 10,000 × *g* supernatant at 100,000 × *g* for 1 hr and resuspension of the resulting pellet (4, 6). One soluble factor is isolated from leukocyte homogenates by protein precipitation at 60–90% ammonium sulfate saturation, and the second is present in the nonadherent protein (pass-through fraction) from the first Mono Q step of the 5-lipoxygenase purification procedure. These factors will be designated pellet, 60–90% ppt, and MQ-PTF, respectively. Details of their preparation have been presented (4).

Assay of 5-Lipoxygenase. 5-Lipoxygenase activity was assayed at 37°C and pH 7.5 for an incubation period of 10 min

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Abbreviations: LT, leukotriene; HPETE, hydroperoxyicosatetraenoic acid; PG, prostaglandin.

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with 100 nmol of [^{14}C]arachidonic acid (1.5 mCi/mmol; 1 Ci = 37 GBq) in a total volume of 1 ml. Assay mixtures contained 2 mM ATP, 3 mM $CaCl_2$, and 20 μM mixed, nonenzymatically generated HPETEs as stimulatory factors (4). Details of the assay conditions and the analysis of samples by extraction, silicic acid column chromatography, and HPLC have been published (4). One unit of enzyme activity produces 1 nmol of 5-HPETE under the standard assay conditions.

Assay of LTA_4 Synthase. 5-HPETE was prepared from the incubation of arachidonic acid with partially purified preparations of 5-lipoxygenase from potatoes (9). 5-HPETE was purified by diethylether extraction and silicic acid column chromatography (10) and was stored at −20°C under an argon atmosphere in the chromatography eluting solvent (hexane/diethylether, 70:30). Prior to assay, the desired quantity of 5-HPETE solution was evaporated to dryness and dissolved in ethanol to give a final concentration of 20 mM as determined by ultraviolet spectroscopy at 234 nm (ε = 28,000).

Routinely, assay samples contained 200 μl of 0.5 M Tris·HCl/10 mM ATP/15 mM $CaCl_2$/4 mM dithiothreitol, pH 8.8, the enzyme sample with desired stimulatory factors, and enough 20 mM potassium phosphate buffer/2 mM EDTA/1 mM dithiothreitol, pH 7.1, to bring the total volume to 1 ml (final pH, 8.4). Samples were warmed to 37°C, and the reaction was initiated by the addition of 5 μl of 5-HPETE solution (100 nmol). After incubation for 10 min, samples were placed on ice, and 2 ml of cold ethanol containing 1 nmol of prostaglandin B_1 (PGB_1) was added. Samples were centrifuged at 2400 rpm for 10 min to remove precipitated protein, and the resulting supernatants were combined with 6 ml of 2.5 mM sodium acetate buffer (pH 5.6) with 0.1 M acetic acid to bring the final pH to 5.6 (≈1 ml). Each sample was then applied to a Sep-Pak C_{18} cartridge (Waters Associates, Milford, MA) that had been washed with 10 ml of ethanol and equilibrated with 10 ml of 2.5 mM sodium acetate buffer at pH 5.6. After sample addition, the Sep-Pak cartridges were washed with 20 ml of 2.5 mM sodium acetate buffer/25% ethanol, pH 5.6, and the LTs were then eluted with 20 ml of 2.5 mM sodium acetate buffer/40% ethanol, pH 5.6. The eluting solvent was evaporated to dryness and the samples were dissolved in 500 μl of HPLC solvent (methanol/acetonitrile/water/acetic acid/triethanolamine, 0.5:0.8:1.2:0.025:0.028) at pH 5.6. HPLC analysis was performed on a column (0.5 cm × 10 cm) of 3-μm Nucleosil C_{18} (Macherey & Nagel, Düren, F.R.G.) eluted isocratically at a flow rate of 1 ml/min with the above solvent. The eluent was monitored for absorbance at 270 nm. The synthesis of LTA_4 was estimated from the sum of the heights of the peaks of compound I (retention time, 17.5 min), compound II (retention time, 19.0 min), and LTB_4 (retention time, 21.0 min) divided by the height of the PGB_1 peak (retention time, 13.6 min). Equimolar quantities of the LTs produced peaks of approximately equal height to that of PGB_1. One unit of LTA_4 synthase activity is expressed as the amount of enzyme that could produce 1 nmol of LTA_4 under the standard assay conditions.

Assay of LTA_4 Synthesis from Arachidonic Acid. In some experiments samples were prepared as for the 5-lipoxygenase assay and incubated with arachidonic acid. The reactions were terminated with the addition of 2 ml of ethanol containing 1 nmol of PGB_1, and the samples were processed with the Sep-Pak procedure as for the LTA_4 synthase assay.

Protein Assay. Protein concentrations were determined by the method of Bradford with bovine serum albumin as standard (11).

RESULTS

Effect of Ca^{2+} and ATP on LTA_4 Synthase Activity. Initial studies of LTA_4 synthase in crude leukocyte homogenates (10,000 × *g* supernatants) revealed that Ca^{2+} and ATP were required for maximal enzymatic activity (Fig. 1). LTA_4 synthase was very low in assay samples containing 1.2 mM EDTA unless a minimum of 2 mM Ca^{2+} was added. The enzyme activity was optimized at 2–5 mM added Ca^{2+}. Concentrations of ATP from 0.5 to 4.0 mM produced a progressive enzyme stimulation, which was maximized at ≈3-fold basal levels. Observations very similar to these (to be published elsewhere) have been observed for the stimulation of human leukocyte 5-lipoxygenase activity by Ca^{2+} and ATP. In view of these results and those of earlier studies that had demonstrated LTA_4 synthase activity in the 5-lipoxygenase from potatoes (7) it seemed reasonable to search for LTA_4 synthase activity in purified human 5-lipoxygenase.

Copurification of Leukocyte 5-Lipoxygenase and LTA_4 Synthase Activities. Fig. 2 shows the elution profile from the final three HPLC steps for the purification of 5-lipoxygenase. In all three cases, samples that contained 5-lipoxygenase also contained LTA_4 synthase such that the two activities coeluted exactly. Particularly notable is the elution profile from the first HPLC step on Mono Q (Fig. 2*A*). In this step, the 5-lipoxygenase reproducibly eluted as a bifid peak, and this behavior was also observed for LTA_4 synthase.

Table 1 summarizes and compares the results from the complete purification of 5-lipoxygenase and LTA_4 synthase. The enzyme recovery and increase in specific activity are remarkably similar for the two activities throughout the purification scheme.

Effect of Stimulatory Factors on LTA_4 Synthase Activity. As reported previously (4) and demonstrated in Fig. 3*B*, maximal 5-lipoxygenase activity required the addition of multiple stimulatory factors to purified samples. Thus, samples subsequent to the ammonium sulfate fractionation required the addition of the 60–90% ppt, and samples subsequent to the first HPLC step on Mono Q (Mono Q-10) required the addition of the MQ-PTF. All samples required inclusion of the pellet factor. As shown in Fig. 3*A*, very similar results were obtained for the LTA_4 synthase activity of these

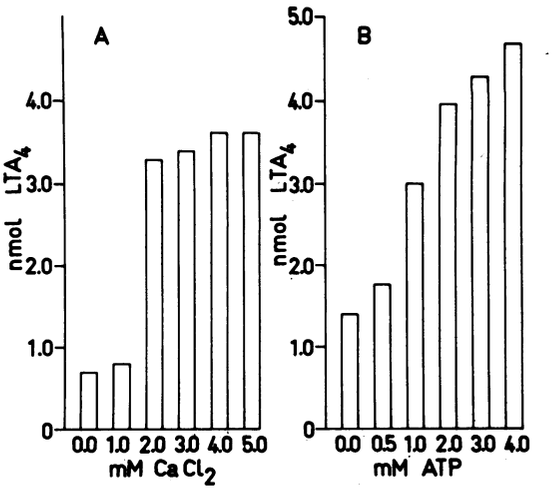

FIG. 1. Effect of Ca^{2+} and ATP concentration on leukocyte LTA_4 synthase activity. (*A*) Six hundred microliters of leukocyte homogenate 10,000 × *g* supernatant was assayed for LTA_4 synthase activity in the presence of 2 mM ATP and the indicated concentrations of Ca^{2+}. (*B*) Conditions were the same as for *A* except that samples contained 3 mM Ca^{2+} and the ATP concentration was varied as indicated. All samples contained 1.2 mM EDTA.

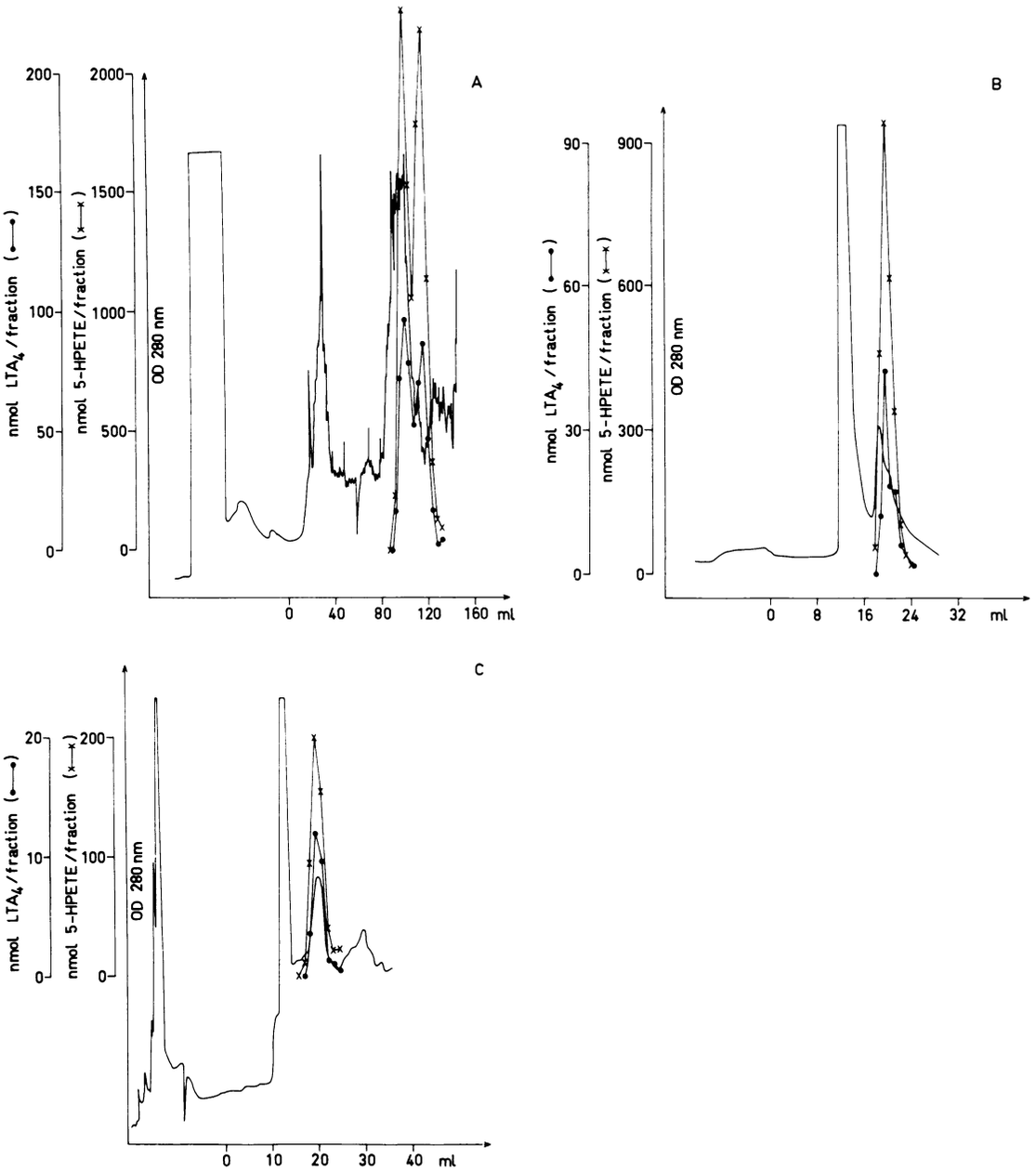

FIG. 2. Coelution of 5-lipoxygenase and LTA_4 synthase activities on HPLC. (*A*) Partially purified 5-lipoxygenase obtained from ammonium sulfate fractionation and gel-filtration chromatography on AcA 44 was applied to a Mono Q HR 10/10 column (Pharmacia) equilibrated in 50 mM Tris·HCl/2 mM EDTA/1 mM dithiothreitol/20 μM ferrous ammonium sulfate/20% glycerol, pH 7.9. Elution was carried out at 4 ml/min with a gradient of 0–0.3 M KCl totaling 240 ml. Fifty-microliter aliquots of the 4-ml fractions were assayed for LTA_4 synthase (•) and 5-lipoxygenase (×) activities in the presence of pellet, 60–90% ppt, and MQ-PTF. The low level of background activity contributed by the stimulatory factors was subtracted from the total activity of each sample. (*B*) Enzyme-containing fractions from the Mono Q HR 10/10 column were applied to a column of hydroxyapatite (Bio-Rad) equilibrated in 10 mM potassium phosphate buffer/0.3 mM $CaCl_2$/1 mM dithiothreitol/20 μM ferrous ammonium sulfate/20% glycerol, pH 7.3. The column was eluted at 0.3 ml/min with a gradient of 0–10% of 500 mM potassium phosphate buffer/6 μM $CaCl_2$/1 mM dithiothreitol/20 μM ferrous ammonium sulfate/20% glycerol, pH 7.3, totaling 10 ml followed by a 10–50% gradient of the same buffer totaling 50 ml. Twenty-five-microliter samples of the 0.9-ml fractions were assayed. (*C*) The enzyme eluted from the hydroxyapatite column was finally purified on a Mono-Q HR 5/5 column (Pharmacia) equilibrated in 25 mM triethanolamine acetate/2 mM EDTA/1 mM dithiothreitol/30% glycerol, pH 7.3. The column was developed at 0.6 ml/min with a gradient of 0–0.4 M sodium acetate totaling 40 ml. Seventy-five-microliter samples of the 1-ml fractions were assayed.

fractions. Thus, it is evident that 5-lipoxygenase and LTA_4 synthase are controlled by the same complex regulatory mechanism.

Production of LTA_4 from Arachidonic Acid by Purified 5-Lipoxygenase. Fig. 4 shows the results of experiments in which various fractions from the 5-lipoxygenase purification scheme were incubated with arachidonic acid under standard conditions for the 5-lipoxygenase assay. Duplicate samples of the reaction mixtures were then analyzed for 5-HPETE and LTA_4 production. The enzyme converted a similar quantity (13–17%) of the endogenously generated 5-HPETE to LTA_4 regardless of the stage of purification. Also shown in Fig. 4 is a comparison of the amount of LTA_4 produced by the enzyme from its endogenously generated 5-HPETE with the amount generated from 100 nmol of exogenously added 5-HPETE under the conditions of the LTA_4 synthase assay. It is clear that the synthesis of LTA_4 is much more efficient when the enzyme utilizes 5-HPETE endogenously generated from arachidonic acid than when the 5-HPETE is supplied from exogenous sources.

Table 1. Purification of 5-lipoxygenase and LTA_4 synthase activities

Fraction	Volume, ml	Total protein, mg	5-Lipoxygenase: Total activity, units	5-Lipoxygenase: Specific activity, units/mg	5-Lipoxygenase: Yield, %	5-Lipoxygenase: Purification, fold	LTA_4 synthase: Total activity, units	LTA_4 synthase: Specific activity, units/mg	LTA_4 synthase: Yield, %	LTA_4 synthase: Purification, fold
10,000 × *g* supernatant	552	2330	38,800	16.6	100	—	1670	0.718	100	—
30–60% ppt	81.0	841	27,300	32.5	70.4	1.96	1130	1.35	67.7	1.88
AcA 44	30.5	170	13,800	80.9	35.6	4.87	576	3.39	34.4	4.72
Mono Q HR 10/10	10.5	9.19	4,690	512	12.1	30.8	187	20.4	11.2	28.4
Hydroxyapatite	4.5	0.541	769	1420	1.98	85.5	28.5	52.8	1.71	73.5
Mono Q HR 5/5	4.0	0.0386	265	6860	0.683	413	12.5	324	0.748	451

Activities were determined for each fraction in the presence of all applicable stimulatory factors. The activity of the 10,000 × *g* supernatant was estimated from the assay of a 10,000 × *g* supernatant in the presence of the pellet factor. This ensured that the contribution of this factor would be similar for all samples and prevented the underestimation of initial enzyme activity due to the time-dependent loss of the membrane-associated factor from the 10,000 × *g* supernatant (6).

DISCUSSION

The data presented here provide strong evidence that a single protein from human leukocytes possesses 5-lipoxygenase and LTA_4 synthase activities. This conclusion is based on the following observations. (*i*) Both enzyme activities show the same requirement for Ca^{2+} and ATP. (*ii*) The activities are isolated together through a five-step purification scheme, showing similar recoveries and increases in specific activity. (*iii*) The two activities coelute exactly on three different chromatography systems. (*iv*) The two activities show identical requirements for one membrane-associated and two cytosolic, nondialyzable stimulatory factors.

It should be noted that the purified 5-lipoxygenase obtained from the final chromatography on Mono Q HR 5/5 is not absolutely homogenous. $NaDodSO_4$ electrophoresis reveals a single major 80-kDa protein that comprises 93–97% of the total and a 63-kDa protein that is the only prominent impurity (4). Thus, it could not be absolutely excluded that the LTA_4 synthase and 5-lipoxygenase activities reside on two separate proteins. However, when individual fractions from the Mono Q HR 5/5 column were subjected to electrophoresis, it was shown that both of the two enzyme activities eluted coincidently with the 80-kDa protein and clearly preceded the 63-kDa component (data not shown). More importantly, it is highly unlikely that two distinct proteins would exhibit identical chromatographic behavior and also demonstrate the same requirements for a complex assortment of stimulatory factors.

Previous work from this laboratory showed that an enzyme from potatoes that possessed 5- and 8-lipoxygenase activities could synthesize LTA_4 from 5-HPETE (7). This finding was explained on the basis of the abstraction of the 10 D-hydrogen from carbon 10, a step which is required for 8-lipoxygenation and the conversion of 5-HPETE to LTA_4. Comparative inhibition studies suggested that the 5- and 8-lipoxygenase reactions occurred at a single active site on the enzyme (7). Although there are obvious similarities between the human leukocyte and potato 5-lipoxygenases, it is not yet known whether the mechanism for the LTA_4 synthase activity is the same for both enzymes. One notable difference is seen in the fact that the potato enzyme shows a similar pH maximum (6.2) for LTA_4 synthase and 5-lipoxygenase, whereas the human leukocyte enzyme shows optimal 5-lipoxygenase activity at pH 7.5 and LTA_4 synthase activity at pH 8.4. It should be noted, however, that in the case of the human enzyme, these studies were performed with crude homogenates. Therefore, the observed pH maxima may reflect the influence of extrinsic factors, such as the activity of peroxidases, rather than the intrinsic properties of the enzyme itself. Obviously, further studies utilizing both purified enzyme and stimulatory factors are required to clarify these questions.

Recently, Goetze *et al.* reported the purification of 5-lipoxygenase from rat basophilic leukemia cells (5). These authors stated that 5-HPETE was the only product formed from arachidonic acid by their enzyme preparation and that no LTA_4 synthesis could be detected. The reason for this discrepancy in results is not known at present, but there are other striking differences between the human and rat enzyme preparations. Particularly, the purified rat enzyme appears to lack the requirement for ATP and for the multicomponent stimulatory system that is operative in the human leukocyte. Furthermore, activity of the purified rat 5-lipoxygenase requires the presence of a nonspecific lipid preparation such as phosphatidylcholine micelles (120 μg/ml), whereas such preparations are ineffective for the human enzyme and cannot substitute for the microsomal membrane factor (unpublished data).

It is interesting to note that the human leukocyte enzyme produced more LTA_4 from 100 nmol of arachidonic acid than when it utilized 100 nmol of 5-HPETE. This was true in spite of the fact that less 5-HPETE (10–40 nmol) was available for LTA_4 formation when the initial substrate was arachidonic acid. Furthermore, the conditions utilized in the incubations with arachidonic acid (pH 7.5) were not optimal for the direct conversion of 5-HPETE to LTA_4. Nevertheless, it is reasonable that the enzyme should use its own endogenously generated 5-HPETE more efficiently than 5-HPETE that is supplied exogenously. From a practical viewpoint, these observations indicate that the 5-lipoxygenase assay and the LTA_4 synthase assay utilized in these studies underestimate the true potential of the enzyme. The 5-lipoxygenase assay fails to detect the 15% of 5-HPETE that is converted to LTA_4. The LTA_4 synthase assay cannot assess the 1.5- to 3-fold higher capacity of the enzyme to synthesize LTA_4 from endogenously generated 5-HPETE.

The finding that the human leukocyte 5-lipoxygenase and LTA_4 synthase activities reside in a single protein carries important implications with regard to enzyme regulation. The human leukocyte 5-lipoxygenase and LTA_4 synthase are apparently controlled by a complex regulatory system requiring Ca^{2+}, ATP, and multiple intracellular components. Clearly, the key to the understanding of the control of LT biosynthesis lies in the delineation of the function of this regulatory mechanism.

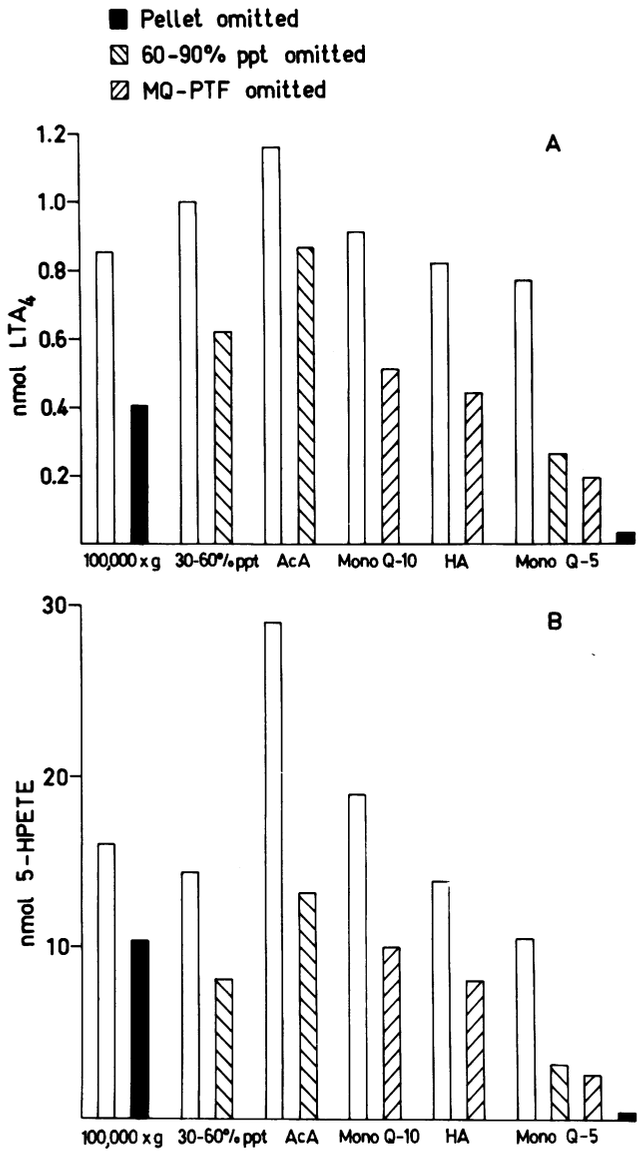

FIG. 3. Stimulatory factor requirements for LTA_4 synthase and 5-lipoxygenase activities. Samples obtained from the various stages in the purification of 5-lipoxygenase were assayed for LTA_4 synthase (*A*) and 5-lipoxygenase (*B*) activities. The samples tested were 100,000 × *g*, a 100,000 × *g* supernatant of leukocyte homogenate (200 μl); 30–60% ppt, the protein precipitating from the leukocyte homogenate at 30–60% saturation of ammonium sulfate (40 μl); AcA, the sample obtained from AcA 44 gel-filtration chromatography of the 30–60% ppt (40 μl); Mono Q-10, the sample obtained by chromatography of the AcA sample on Mono-Q HR 10/10 (40 μl); HA, the fraction obtained from hydroxyapatite HPLC of the Mono Q-10 sample (40 μl); and Mono Q-5, the purified enzyme after chromatography of the hydroxyapatite sample on Mono Q HR 5/5 (80 μl). Unless otherwise indicated by the shading of the bars, samples subsequent to the 30–60% ammonium sulfate precipitation contained 60–90% ppt, samples subsequent to the Mono Q HR 10/10 step contained MQ-PTF, and all samples contained pellet. In each case, low levels of enzyme activity that were present in the stimulatory factors alone were subtracted from the total activity of the samples.

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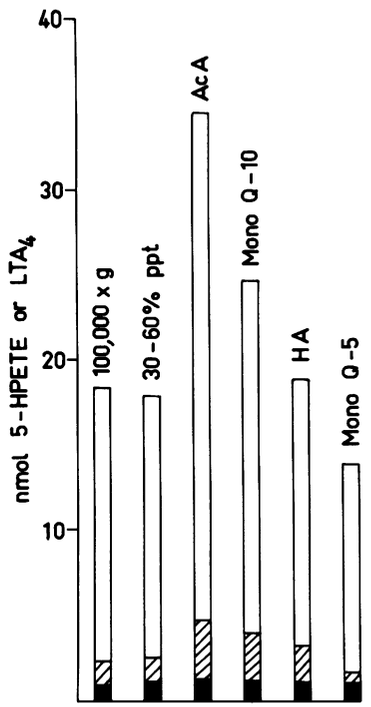

FIG. 4. Synthesis of LTA_4 from endogenously generated 5-HPETE. Duplicate samples prepared from fractions obtained during the purification of 5-lipoxygenase were incubated with 100 nmol of arachidonic acid under the standard conditions for the 5-lipoxygenase assay. One sample of each set of duplicates was then analyzed for 5-HPETE content and the other was analyzed for LTA_4. A third set of samples was also prepared under the conditions for the LTA_4 synthase assay and incubated with 100 nmol of 5-HPETE for analysis of LTA_4 production. In all cases, the appropriate stimulatory factors were included. The total height of each bar represents the amount of 5-HPETE produced from arachidonic acid as calculated from the sum of 5-HPETE and LTA_4 measured in the parallel assays. The total height of the hatched and shaded areas of the bars represents the synthesis of LTA_4 by samples incubated with arachidonic acid. The height of the shaded areas alone indicates the synthesis of LTA_4 by samples incubated with 100 nmol of 5-HPETE. The identity of each sample is indicated above the bars by notations that are defined in the legend to Fig. 3.

1. Samuelsson, B. (1983) *Science* **220**, 568–575.
2. Borgeat, P. & Samuelsson, B. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3213–3217.
3. Hammarström, S., Murphy, R. C., Samuelsson, B., Clark, D. A., Mioskowski, C. & Corey, E. J. (1979) *Biochem. Biophys. Res. Commun.* **91**, 1266–1272.
4. Rouzer, C. A. & Samuelsson, B. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6040–6044.
5. Goetze, A. M., Fayer, L., Bouska, J., Bornemeier, D. & Carter, G. W. (1985) *Prostaglandins* **29**, 689–701.
6. Rouzer, C. A., Shimizu, T. & Samuelsson, B. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 7505–7509.
7. Shimizu, T., Rådmark, O. & Samuelsson, B. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 689–693.
8. Rådmark, P., Shimizu, T., Fitzpatrick, F. & Samuelsson, B. (1984) *Biochim. Biophys. Acta* **792**, 324–329.
9. Corey, E. J., Albright, J. O., Barton, A. E. & Hashimoto, S. (1980) *J. Am. Chem. Soc.* **102**, 1435–1436.
10. Borgeat, P. & Samuelsson, B. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 2148–2152.
11. Bradford, M. M. (1976) *Anal. Biochem.* **72**, 248–254.