Proc. Natl. Acad. Sci. USA
Vol. 77, No. 1, pp. 308–312, January 1980
Biochemistry

Arachidonate metabolism via lipoxygenase and 12*L*-hydroperoxy-5,8,10,14-icosatetraenoic acid peroxidase sensitive to anti-inflammatory drugs

(aspirin/salicylate/indomethacin/acetaminophen/human platelets)

MARVIN I. SIEGEL*, RANDY T. MCCONNELL*, NED A. PORTER†, AND PEDRO CUATRECASAS*

*Molecular Biology Department, The Wellcome Research Laboratories, Research Triangle Park, North Carolina 27709; and the †Paul M. Gross Chemical Laboratories, Duke University, Durham, North Carolina 27706

Communicated by George H. Hitchings, October 29, 1979

ABSTRACT **The enzymes of arachidonate metabolism via the lipoxygenase pathway in human platelet cytosol have been characterized and partially purified. The lipoxygenase activity has a pH optimum of 7.3 and reaches half-maximal activity at an arachidonate concentration of 80 μM. The oxidation of arachidonate by these enzymes is inhibited by reagents that modify sulfhydryl groups. Two separable lipoxygenase activities can be detected by chromatography of platelet cytosol on Sephadex G-150 and of partially purified preparations on DEAE-Sephadex. One of these has an apparent M_r of 100,000. A second enzyme species behaves as a M_r 160,000 entity containing, in addition to lipoxygenase, a peroxidase activity that catalyzes the conversion of 12*L*-hydroperoxy-5,8,10,14-icosatetraenoic acid (HPETE) to 12*L*-hydroxy-5,8,10,14-icosatetraenoic acid (HETE). Aspirin, indomethacin, sodium salicylate, phenylbutazone, ibuprofen, naproxen, and sulindac, but not acetaminophen or phenacetin, give rise to increased levels of HPETE in the lipoxygenase pathway. This increase in HPETE levels is the result of the ability of these drugs to inhibit directly the enzymatic conversion of HPETE to HETE.**

Human platelets can metabolize exogenously added arachidonate to 12*L*-hydroxy-5,8,10,14-icosatetraenoic acid (HETE) as well as to prostaglandin synthetase (fatty acid cyclo-oxygenase) products (1). In the presence of concentrations of aspirin or indomethacin that totally inhibit cyclo-oxygenase, platelet suspensions synthesize increased amounts of HETE from arachidonate, thereby indicating that the lipoxygenase pathway is distinct from cyclo-oxygenase activity (2). Moreover, when horse platelets are stimulated by thrombin or other aggregating agents, nearly 75% of the arachidonate released from endogenous sources by the action of phospholipases is metabolized via lipoxygenase (3, 4), suggesting some important function for these arachidonate metabolites.

The possible biological roles of the products of the metabolism of arachidonate via lipoxygenase have not been elucidated. However, HETE, the end product of this metabolic route in human platelets (1, 5), may have chemotactic activity for human polymorphonuclear leukocytes (6, 7). Moreover, a lipid fraction from *Escherichia coli* that is chemically and chromatographically similar to HETE has chemotactic activity with human polymorphs and rabbit alveolar macrophages (8). In addition, the labile lipoxygenase product (5), 12*L*-hydroperoxy-5,8,10,14-icosatetraenoic acid (HPETE), may have important, as yet unrecognized, biological functions. For example, the release of anaphylactic mediators from guinea pig lung is increased by hydroperoxy fatty acids (9, 10). Furthermore, various fatty acid hydroperoxides which are analogs of HPETE inhibit the formation of prostacyclin via prostacyclin synthetase in porcine aorta (11) and other tissues (12).

The anti-inflammatory, analgesic, and antipyretic drugs aspirin and indomethacin inhibit cyclo-oxygenase *in vitro* (13, 14) whereas sodium salicylate does not (13, 15, 16) despite the fact that it is an active drug when administered *in vivo* (17–21). However, all three drugs have recently been shown to interfere with arachidonate metabolism via lipoxygenase in various cell types by reversibly inhibiting the conversion of HPETE to HETE (22), suggesting that this effect may contribute to the mechanism of action of these drugs. Because these drugs share a common site of action and because this inhibition by aspirin-like drugs suggests the presence of a specific HPETE peroxidase activity, it was of special interest to investigate further the metabolism of arachidonate via lipoxygenase. To this end, the enzymes of this metabolic route in human platelets have been partially characterized and purified and the effects of some non-aspirin-like analgesic, antipyretic, or anti-inflammatory drugs have been examined.

MATERIALS AND METHODS

Arachidonic acid was obtained from P-L Biochemicals. [1-^{14}C]Arachidonate (57 mCi/mmol; 1 Ci = 3.7×10^{10} becquerels) was from Amersham. Precoated thin-layer chromatography plates, Sil G-25 without gypsum, were from Brinkmann Instruments. Burroughs Wellcome provided aspirin, acetaminophen, and phenacetin. Clinoril (sulindac) and indomethacin were from Merck, Sharp and Dohme, Motrin (ibuprofen) was from Upjohn, Naprosyn (naproxen) was from Syntex, and phenylbutazone was from Ciba-Geigy.

Purification of the Enzymes of Arachidonate Metabolism Via Lipoxygenase. One unit of human blood from subjects who had received no aspirin-like compounds during the preceding 4 weeks was collected in 0.15 vol of the anticoagulant citrate/dextrose. Platelets were then collected by differential centrifugation. Whole blood was centrifuged for 20 min at 300 × *g* at room temperature and the platelet-containing plasma was withdrawn from above the pelleted erythrocytes. After addition of EDTA (to a final concentration of 1 mM), the platelet-containing plasma was cooled to 0°C and centrifuged at 2000 × *g* for 20 min. The pelleted platelets were resuspended and lysed by freeze-thawing three times in 25 mM Tris·HCl at pH 7.7. Platelet cytosol was prepared from the frozen-thawed homogenate by centrifugation at 0° for 1 hr at 100,000 × *g* in a type 65 rotor (Beckman) in a Sorvall OTD-2. Solid ammonium sulfate was added to the resulting supernatant to a final concentration of 30% saturation, and the precipitated material was

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: HETE, 12*L*-hydroxy-5,8,10,14-icosatetraenoic acid; HPETE, 12*L*-hydroperoxy-5,8,10,14-icosatetraenoic acid.

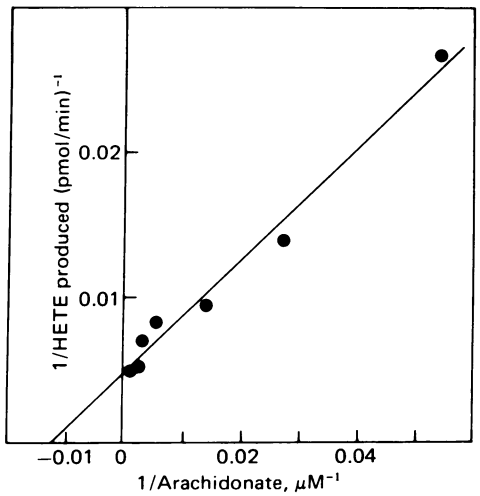

FIG. 1. Dependence of the lipoxygenase enzymes on the concentration of arachidonate. Two hundred micrograms of platelet 100,000 × *g* supernatant protein was assayed.

removed by centrifugation at 20,000 × *g* at 0°C in a Sorval RC-5B. Additional ammonium sulfate was added to the supernatant to a final concentration of 70% saturation, and the precipitate was collected by centrifugation as described above. The pellet was dissolved in a minimal volume of 25 mM Tris·HCl at pH 7.7 and was dialyzed against the same buffer for 5 hr. The dialyzed enzyme was adjusted to pH 6 with 0.1 M HCl and centrifuged. The supernatant was readjusted to pH 7.7 with 0.1 M NaOH. This solution served as the starting material for further purification.

Assay of the Enzymes of Arachidonate Metabolism Via Lipoxygenase. Assays were conducted in 25 mM Tris·HCl (pH 7.4) at 37°C unless otherwise noted. The 0.1-ml reaction mixtures containing various concentrations of [1-^{14}C]arachidonate were initiated by the addition of the enzyme solution. In order to terminate the assay, 2.4 ml of chloroform/methanol, 1:1 (vol/vol), and 0.9 ml of 0.1% formic acid were added, and the suspension was vortexed and immediately cooled in ice. After centrifugation at 500 × *g* for 10 min at 0°C, the organic layer was withdrawn and evaporated under dry N_2. The residue was redissolved in a minimal volume of chloroform/methanol, 1:2 (vol/vol), and spotted on silica thin-layer chromatography plates. Chromatograms were developed with an ascending solvent consisting of ligroine/diethyl ether/glacial acetic acid, 50:50:1 (vol/vol). Products were located by autoradiography and the appropriate regions of the plates were scraped off and assayed for radioactivity.

Identification of HPETE and HETE as Reaction Products. The two products formed from arachidonate by the action of the enzymes of the lipoxygenase pathway were located by autoradiography after thin-layer chromatography as described above. The one migrating with an R_F of 0.33 cochromatographed with biologically produced HETE identified previously (9, 10). The second product had an R_F of 0.37. After reduction with sodium borohydride in methanol, this product cochromatographed with HETE. When reaction mixtures were subjected to high-pressure liquid chromatography on a Spectra Physics 3500 HPLC equipped with a Spectra Physics 4.6 mm × 25 cm 5-μm Spheresorb silica column and eluted with hexane/2-propanol/glacial acetic acid, 991:8:1 (vol/vol) at 1 ml/min, two products were observed. The first, eluting with a retention time of 18 min, cochromatographed with HETE on thin-layer chromatography and yielded a gas chromatograph/mass spectrum characteristic of HETE (23). The second, eluting at 28 min, cochromatographed on thin-layer chromatography with the putative HPETE identified above and with chemically synthesized 12-HPETE (24) on high-pressure liquid chromatography and thin-layer chromatography. After reduction with triphenylphosphine, this compound cochromatographed with HETE in thin-layer and high-pressure chromatography systems and yielded the same gas chromatograph/mass spectrum described above. Finally, addition of the putative HPETE to reaction mixtures containing lipoxygenase and peroxidase activities gave rise to a product cochromatographing with HETE. Therefore, these data are consistent with this reducible compound being HPETE.

Table 1. Effect of sulfhydryl reagents on human lipoxygenase activity

Treatment	Lipoxygenase activity, %
None	100
Iodoacetamide	7
N-Ethylmaleimide	23
p-Chloromercuriphenylsulfonate	23

Human platelet 100,000 × *g* supernatant was incubated for 1 hr at 0°C with 1 mM iodoacetamide, *N*-ethylmaleimide, or *p*-chloromercuriphenylsulfonate in 25 mM Tris·HCl (pH 7.7) and then dialyzed overnight against 25 mM Tris·HCl (pH 7.7). Aliquots were assayed with 200 μM [1-^{14}C]arachidonate.

RESULTS

Characterization of the Lipoxygenase System. When platelets prepared by differential centrifugation from freshly drawn human blood were lysed in hypotonic medium by repetitive freeze-thawing, >60% of the lipoxygenase activity appeared in the 100,000 × *g* supernatant (269 nmol/min total activity in the supernatant vs. 161 nmol/min in the pellet from 1 g of platelet protein). The presence of lipoxygenase activity in the cytosol is consistent with the known localization of this enzyme in bovine platelets (5). The activity remaining in the particulate fraction may be the same as that reported by Ho *et al.* (23) but has not yet been investigated further. Metabolism of arachidonate by the cyclo-oxygenase pathway was not observed in the platelet supernatant (data not shown).

The production of HPETE and HETE by the sequential lipoxygenase and peroxidase activities of human platelet 100,000 × *g* supernatant was linear for at least 4 min at 37°C (data not shown). The oxidation of arachidonate was linear with protein concentrations up to 3 mg/ml (data not shown). As with the particulate lipoxygenase activity (23), the cytosolic enzymes were active over a broad range of pH with an optimum at 7.3 (data not shown). The concentration of substrate necessary to achieve half-maximal oxidation of arachidonate by lipoxygenase was approximately 80 μM (Fig. 1).

The oxidation of arachidonate via lipoxygenase was inhibited by reagents that modify sulfhydryl moieties. Incubation of the enzyme preparation with 1 mM iodoacetamide, *N*-ethylmaleimide, or *p*-chloromercuriphenylsulfonate led to loss of activity, suggesting the presence of essential sulfhydryl groups (Table 1). In addition, dialysis of lipoxygenase preparations against 50 mM EDTA, pH 7.6/1 mM dithiothreitol, but not against 50 mM Tris·HCl, pH 7.6/1 mM dithiothreitol, led to a loss of >90% of the activity (data not shown), suggesting that a metal ion may also be necessary for enzymatic activity.

Resolution and Partial Purification. When cytosol from frozen-thawed human platelets was chromatographed on a Sephadex G-150 column, two peaks of lipoxygenase activity were observed (Fig. 2). One of these eluted with an apparent

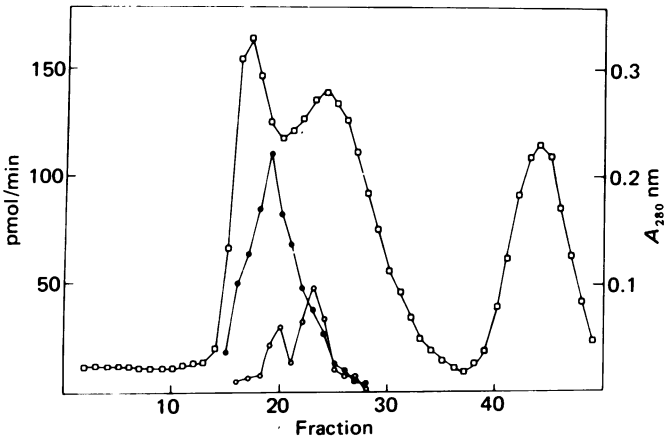

FIG. 2. Gel filtration of platelet cytosol. Platelet 100,000 × *g* supernatant (0.5 ml) was applied to a 0.9 × 50 cm Sephadex G-150 column previously equilibrated with 25 mM Tris·HCl, pH 7.7/0.1 M NaCl; 1.0-ml fractions were collected. Aliquots were assayed with 200 μM [1-^{14}C]arachidonate. □, $A_{280\ nm}$; ○, HPETE; ●, HETE.

M_r of approximately 100,000 and produced predominantly [1-^{14}C]HPETE when assayed with [1-^{14}C]arachidonate. The other protein peak eluted with an estimated M_r of 160,000 and produced HPETE and much larger amounts of HETE. Because the production of HETE from arachidonate presumably requires the prior synthesis of HPETE (5), this protein fraction from the Sephadex column must contain a lipoxygenase species and a peroxidase activity capable of converting the hydroperoxy to the hydroxy fatty acid. On the other hand, it is possible that the peak of estimated M_r 160,000 could represent a complex between the lipoxygenase and a protein that is not a peroxidase but that provides the reducing equivalents for a putative peroxidase activity inherent in the lipoxygenase. However, when column fractions were assayed in the presence of 0.13 mM guaiacol, exactly the same pattern of HPETE and HETE formation as illustrated in Fig. 2 was obtained (data not shown). This supports the hypothesis that the lack of significant peroxidase activity in the M_r 100,000 fraction is due to lack of enzyme and not to a lack of reducing agent.

Lipoxygenase activity can be partially purified from lysed human platelet cytosol by ammonium sulfate fractionation, acid precipitation, and column chromatography. Application of the neutralized pH 6 supernatant to a DEAE-Sephadex column followed by elution with a linear KCl gradient yielded two peaks of lipoxygenase activity (Fig. 3). The protein eluting at lower ionic strength contained both lipoxygenase and peroxidase activities because incubation of these fractions with [1-^{14}C]arachidonate resulted in the formation of [1-^{14}C]HPETE and larger amounts of [1-^{14}C]HETE. These enzymatic activities, therefore, correspond to those of the M_r 160,000 fraction observed upon gel filtration of the platelet cytosol. A second peak of lipoxygenase containing little or no peroxidase activity (that is, high HPETE but relatively low HETE production) was eluted at higher KCl concentrations. This activity was similar to that of the M_r 100,000 fraction from Sephadex G-150.

A summary of the partial purification of these two protein species is shown in Table 2. The lipoxygenase activity in the peak eluting at higher ionic strength (peak 2) was purified approximately 100-fold and the fraction containing lipoxygenase and peroxidase activities was enriched nearly 25-fold in comparison to human platelet homogenates.

Effect of Anti-Inflammatory, Analgesic, and Antipyretic Drugs. Since Siegel *et al.* (22) reported that aspirin, indomethacin, and sodium salicylate appear to interfere with arachidonate metabolism via the lipoxygenase pathway by inhibiting conversion of HPETE to HETE in human platelets and other tissues, it became of interest to investigate whether the non-aspirin-like drugs phenylbutazone, acetaminophen, phenacetin, ibuprofen, naproxen, and sulindac have similar effects. When the metabolism of arachidonate in human platelet cytosol containing lipoxygenase and peroxidase activities was measured in the presence of various concentrations of phenylbutazone, there was an increase in the amount of HPETE and a relative decrease in the amount of HETE produced (Fig. 4). The concentration of phenylbutazone necessary for the half-maximal increase in HPETE concentration was approximately 50 μM. However, whereas aspirin, salicylate, indomethacin, and phenylbutazone similarly affect the metabolism of arachidonate via lipoxygenase, acetaminophen and phenacetin appear to be inactive in this system (Table 3).

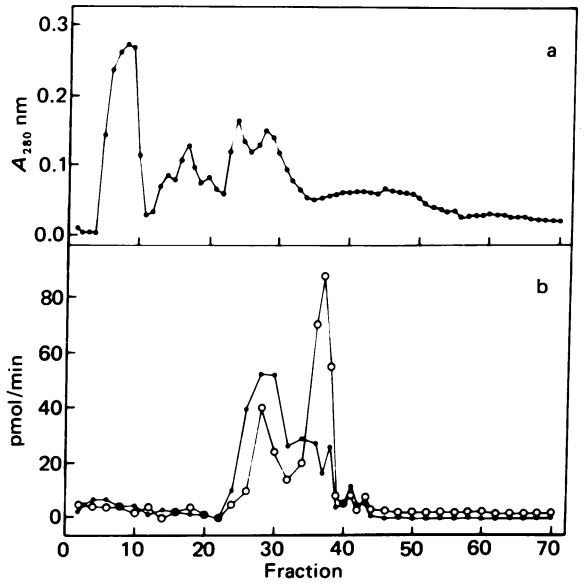

FIG. 3. Purification of the lipoxygenase enzymes on DEAE-Sephadex. The neutralized supernatant from the acid precipitation step of the purification scheme (see Table 2) was applied to a 1.5 × 30 cm DEAE-Sephadex A-25 column. Elution was achieved with a linear gradient from 0 to 0.5 M KCl in 25 mM Tris·HCl (pH 7.7). Fractions (5 ml) were assayed with 200 μM [1-^{14}C]arachidonate. (*a*) $A_{280\ nm}$ profile. (*b*) ○, HPETE; ●, HETE.

The more recently introduced propionic acid derivatives ibuprofen and naproxen are inhibitors of prostaglandin synthetase (25). In addition, the acetic acid derivative sulindac is converted *in vivo* to a metabolically active cyclo-oxygenase inhibitor (25). However, as observed with aspirin-like drugs and phenylbutazone, these anti-inflammatory drugs also increased HPETE levels (Table 3). The relatively high sulindac concen-

Table 2. Purification of human platelet lipoxygenases

Fraction	Total protein, mg	Activity: Total, nmol/min	Activity: Specific, nmol/min/mg	Recovery
Homogenate	888	190	0.2	1.00
Cytosol	247	269	1.1	1.42
30–70% $(NH_4)_2SO_4$	71	168	2.4	0.88
pH 6 supernatant	43	136	3.2	0.72
DEAE-Sephadex:				
Peak 1 (lipoxygenase/peroxidase)	6.4	35.8	5.6	0.19
Peak 2 (lipoxygenase)	2.3	50.4	21.9	0.27

All assays contained 200 μM [1-^{14}C]arachidonate.

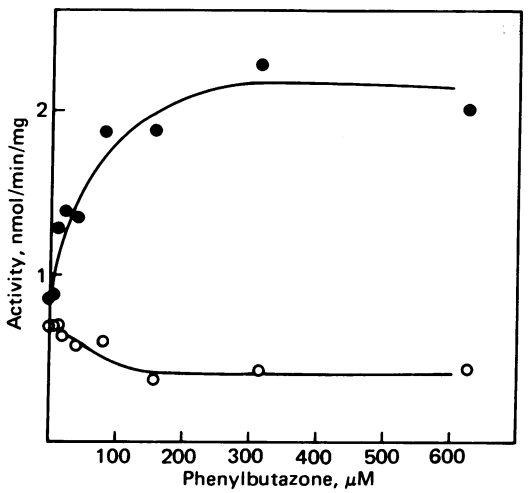

FIG. 4. Effect of phenylbutazone on arachidonate metabolism via lipoxygenase. Human platelet 100,000 × *g* supernatant (100 μg of protein) was assayed with 80 μM [1-^{14}C]arachidonate and various concentrations of phenylbutazone in 50 mM Tris·HCl at pH 7.4. Values are ±5%. ●, HPETE; ○, HETE.

tration required for this effect probably reflects the lack of conversion to its active metabolite in this system.

To investigate whether aspirin-like drugs directly inhibit the conversion of HPETE to HETE, lysed platelet 100,000 × *g* supernatants were incubated with chemically synthesized (24) [1-^{14}C]HPETE at 2 μM. Whereas <10% of the added HPETE was recovered after a 1-min incubation at 37°C, nearly 22% of the hydroperoxy fatty acid remained when assays contained sodium salicylate or aspirin and 65% when indomethacin was present (Fig. 5).

Anti-inflammatory drugs inhibited the conversion of HPETE to HETE catalyzed by partially purified preparations of platelet lipoxygenase pathway enzymes as well as by platelet supernatants. Assay of enzyme preparations obtained after Sephadex G-150 chromatography of 100,000 × *g* supernatants from lysed human platelets confirmed the sensitivity of HPETE peroxidase activity to indomethacin inhibition. In the absence of drug, fractions containing both lipoxygenase and peroxidase activities converted arachidonate to HPETE and larger amounts of HETE (Figs. 2 and 6). However, when these same fractions were assayed in the presence of indomethacin, there was a marked increase in HPETE recovered and a relative decrease in HETE, indicating that HPETE peroxidase is inhibited (Fig. 6). The marked increase in total lipoxygenase activity in the presence of indomethacin is probably due to the fact that HPETE is a potent activator of platelet lipoxygenase (26).

Table 3. Inhibition of HPETE peroxidase activity by anti-inflammatory, analgesic, or antipyretic drugs

Addition*	HPETE/(HPETE + HETE)
None	0.31
2 mM aspirin (500 μM)*	0.81
2 mM sodium salicylate (100 μM)	0.98
200 μM indomethacin (25 μM)	0.60
156 μM phenylbutazone (50 μM)	0.80
5 mM acetaminophen	0.38
5 mM phenacetin	0.28
500 μM ibuprofen (200 μM)	0.52
500 μM naproxen (200 μM)	0.67
2.5 mM sulindac (1 mM)	0.69

Human platelet 100,000 × *g* supernatant was assayed in the presence of the various drugs and 80 μM [1-^{14}C]arachidonate in 50 mM Tris·HCl at pH 7.4. All ratios are ±0.05.

* Concentration for half-maximal effect (ED_{50}) is in parentheses.

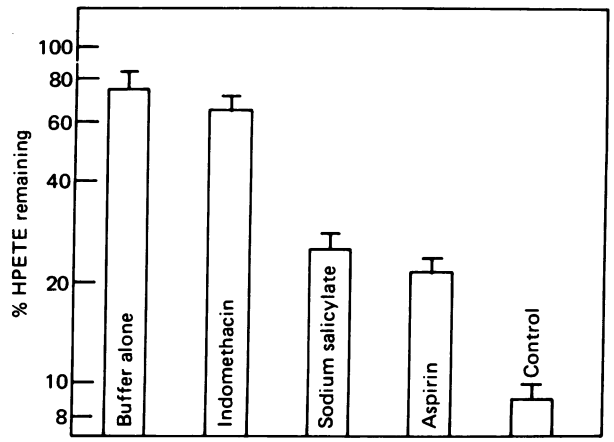

FIG. 5. Effect of aspirin-like drugs on the conversion of HPETE to HETE. [1-^{14}C]HPETE at 2.4 μM was incubated in 1.0 ml of 50 mM Tris·HCl (pH 7.4) containing lysed human platelet 100,000 × *g* supernatant (0.90 mg of protein) for 1 min at 37°C in the absence or presence of 2 mM sodium salicylate, 2 mM aspirin, or 250 μM indomethacin. Three separate experiments were performed with assays in duplicate. Recovery of radioactivity was constant in the presence of the various agents. Incubations containing boiled platelet preparations gave the same results as those with buffer alone.

DISCUSSION

One of the more interesting observations of the present studies is the appearance of two proteins containing lipoxygenase activity in human platelet 100,000 × *g* supernatants (Figs. 2 and 3). One of these activities appears to copurify with a peroxidase activity capable of converting HPETE to HETE. The fact that the lipoxygenase and peroxidase activities purify together on Sephadex G-150 (Fig. 2) and DEAE-Sephadex (Fig. 3) suggests that they might be components of the same M_r 160,000 protein acting as a functional complex, but more evidence is necessary to establish such a complex. In addition, the fact that aspirin, indomethacin, sodium salicylate, phenylbutazone, ibuprofen, naproxen, and sulindac inhibit the conversion of HPETE to HETE (Figs. 4–6; Table 3; ref. 22) further supports the conclusion that an enzyme with HPETE peroxidase activity exists in human platelets. Because human glutathione peroxidase is not inhibited by aspirin and indomethacin (H. White, personal communication), it is not a likely candidate for the source of this peroxidase activity.

The origin of the M_r 100,000 lipoxygenase species is not known. It may have arisen by separation from a cytosolic complex of lipoxygenase and peroxidase activities prior to column chromatography, by liberation from human platelet microsomes during lysis by freeze-thawing, or by proteolysis. Attempts to isolate an HPETE peroxidase have been severely hampered by limited supplies of [1-^{14}C]HPETE.

Because of the similarity of drugs on HPETE peroxidase activity, it is tempting to extrapolate from the platelet model and speculate that at least some of the pharmacological actions of these drugs in tissues other than platelets may be related to this perturbation of arachidonate metabolism via the lipoxygenase pathway. For example, because prostacyclin appears to be hyperalgesic (‡, 27) and HPETE analogs inhibit prostacyclin synthetase (11, 12, 28), some of the activity of these drugs against the hyperalgesia caused by inflammation may be explained by their common ability to increase levels of HPETE, even though some hydroperoxides may be hyperalgesic at high concentrations (29). Although certain hydroperoxides may

‡ Ferreira, S. H. (1978) *Proceedings of the Seventh International Congress of Pharmacologists, Paris, 420 (Abstr.).*

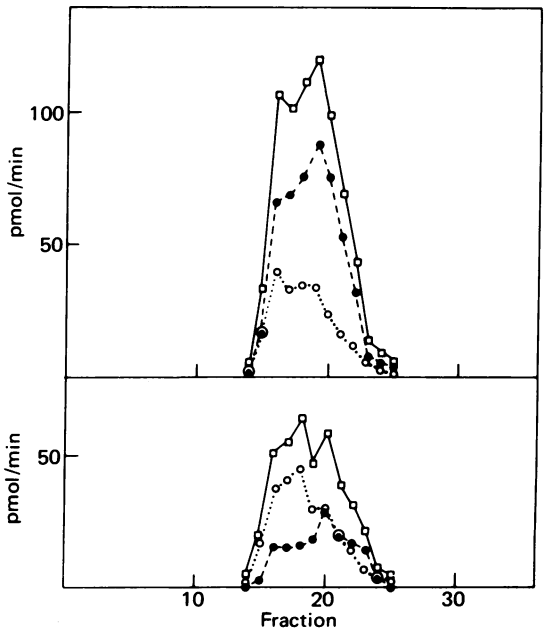

FIG. 6. Effect of indomethacin on a partially purified lipoxygenase/peroxidase preparation. Platelet cytosol was chromatographed on a Sephadex G-150 column as described in Fig. 2. Aliquots were assayed with 80 μM [1-^{14}C]arachidonate in the presence (*Upper*) or absence (*Lower*) of 200 μM indomethacin. ●, HPETE; ○, HETE; □, HPETE plus HETE.

activate cyclo-oxygenase (30), low concentrations of HPETE inhibit human platelet prostaglandin and thromboxane production from arachidonate (26). Therefore, the ability of sodium salicylate to inhibit prostaglandin production *in vivo* (18, 21) but not *in vitro* (13, 15, 16) may be explained by this drug's ability to increase HPETE-levels. Moreover, because hydroperoxy fatty acids enhance the release of anaphylactic mediators from perfused guinea pig lungs (9, 10), the acute allergic responses to aspirin-like drugs (31) may be the result of these patients' extreme sensitivity to HPETE peroxidase inhibition or the resultant increase in HPETE.

HETE appears to be chemotactic (6–8). Therefore, inhibition of HETE production may be in part related to some of these drugs' anti-inflammatory activity [i.e., blockage of recruitment of phagocytic cells (32, 33)].

Because the physiological and pharmacological properties of HPETE or its potential metabolites (34–36) are largely unknown, it is difficult to speculate on the possible consequences of increased levels. However, it is notable that acetaminophen and phenacetin, whose mechanisms of action have not yet been elucidated (37–40), do not affect arachidonate metabolism via lipoxygenase (Table 3).

It will be important to study the roles of HPETE and HETE in normal and pathological states. As the functions of these compounds are elucidated, it may be possible to explain the biochemical basis and relative importance of these lipoxygenase products, as well as cyclo-oxygenase metabolites, in the action of anti-inflammatory, analgesic, and antipyretic drugs.

We thank Drs. H. White, R. Vinegar, E. Lapetina, and K. Chandrabose for valuable discussions.

1. Hamberg, M. & Samuelsson, B. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3400–3404.
2. Hamberg, M., Svensson, J. & Samuelsson, B. (1974) *Proc. Natl. Acad. Sci. USA* **71**, 3824–3828.
3. Lapetina, E. G., Schmitges, C. J., Chandrabose, K. & Cuatrecasas, P. (1977) *Biochem. Biophys. Res. Commun.* **76**, 828–835.
4. Lapetina, E. G., Chandrabose, K. & Cuatrecasas, P. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 818–822.
5. Nugteren, D. H. (1975) *Biochim. Biophys. Acta* **380**, 299–307.
6. Turner, S. R., Tainer, J. A. & Lynn, W. S. (1976) *Nature (London)* **257**, 680–681.
7. Goetzl, E. J., Woods, J. M. & Gorman, R. R. (1977) *J. Clin. Invest.* **59**, 179–183.
8. Tainer, J. A., Turner, S. R. & Lynn, W. S. (1975) *Am. J. Pathol.* **81**, 401–410.
9. Adcock, J. J., Garland, L. G., Moncada, S. & Salmon, J. A. (1978) *Prostaglandins* **16**, 179–187.
10. Adcock, J. J., Garland, L. G., Moncada, S. & Salmon, J. A. (1978) *Prostaglandins* **16**, 163–177.
11. Salmon, J. A., Smith, D. R., Flower, R. J., Moncada, S. & Vane, J. R. (1978) *Biochim. Biophys. Acta* **523**, 250–262.
12. Moncada, S., Gryglewski, R. J., Bunting, S. & Vane, J. R. (1976) *Prostaglandins* **12**, 715–737.
13. Vane, J. R. (1971) *Nature (London) New Biol.* **231**, 232–235.
14. Ferreira, S. H., Moncada, S. & Vane, J. R. (1971) *Nature (London) New Biol.* **231**, 237–239.
15. Smith, J. B. & Willis, A. L. (1971) *Nature (London) New Biol.* **231**, 235–237.
16. Vargaftig, B. B. (1978) *J. Pharm. Pharmacol.* **30**, 101–104.
17. Smith, M. J. H., Ford-Hutchinson, A. W. & Elliot, P. N. C. (1975) *J. Pharm. Pharmacol.* **27**, 473–478.
18. Hamberg, M. (1972) *Biochem. Biophys. Res. Commun.* **49**, 720–726.
19. Willis, A. L., Davison, P., Ramwell, P. W., Brocklehurst, W. E. & Smith, B. (1972) *Prostaglandins in Cellular Biology*, eds. Ramwell, P. W. & Pharris, B. B. (Plenum, New York), pp. 227–259.
20. Collier, H. O. J. (1969) *Adv. Pharmacol. Chemother.* **7**, 333–405.
21. Higgs, G. A., Harvey, E. A., Ferreira, S. H. & Vane, J. R. (1976) *Adv. Prost. Thromboxane Res.* **1**, 105–110.
22. Siegel, M. I., McConnell, R. T. & Cuatrecasas, P. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 3774–3778.
23. Ho, P. P. K., Walters, C. P. & Sullivan, H. R. (1977) *Biochem. Biophys. Res. Commun.* **76**, 398–405.
24. Porter, N. A., Wolf, R. A., Yarbro, E. M. & Weenen, H. (1979) *Biochem. Biophys. Res. Commun.* **89**, 1058–1064.
25. Lombardino, J. G. (1978) *Annu. Rep. in Med. Chem.* **13**, 167–178.
26. Siegel, M. I., McConnell, R. T., Porter, N. A. & Cuatrecasas, P. (1979) *Biochem. Biophys. Res. Commun.* **89**, 1273–1280.
27. Komoriya, K., Ohmari, H., Azuma, A., Kurozumi, S. & Hashimoto, Y. (1978) *Prostaglandins* **15**, 557–564.
28. Ham, E. A., Egan, R. W., Soderman, D. D., Gale, P. H. & Kuehl, F. A. (1979) *J. Biol. Chem.* **254**, 2191–2194.
29. Ferreira, S. H. (1972) *Nature (London) New Biol.* **240**, 200–203.
30. Hemler, M. E., Graff, G. & Lands, W. E. M. (1978) *Biochem. Biophys. Res. Commun.* **85**, 1325–1331.
31. Martinelli, N. A. & Usandivaras, G. (1977) *Thorax* **32**, 684–690.
32. Vinegar, R., Truax, J. F. & Selph, J. L. (1976) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **35**, 2447–2456.
33. Vinegar, R., Truax, J. F. & Selph, J. L. (1973) *Proc. Soc. Exp. Biol. Med.* **143**, 711–714.
34. Bryant, R. W. & Bailey, J. M. (1979) *Prostaglandins* **17**, 9–18.
35. Jones, J. L., Kerry, P. J., Poyser, N. L., Walker, I. C. & Wilson, N. H. (1978) *Prostaglandins* **16**, 583–589.
36. Bild, G. S., Bhat, S. G., Axelrod, B. & Iatridis, P. G. (1978) *Prostaglandins* **16**, 795–801.
37. Vinegar, R., Truax, J. F. & Selph, J. L. (1976) *Eur. J. Pharmacol.* **37**, 23–30.
38. Glenn, E. M., Bowman, B. J. & Rohloff, N. A. (1977) *Agents and Actions* **7**, 513–516.
39. Flower, R. L. & Vane, J. R. (1972) *Nature (London) New Biol.* **240**, 410–411.
40. Zenser, T. V., Mattammal, M. B., Herman, C. A., Joshi, S. & Davis, B. B. (1978) *Biochim. Biophys. Acta* **542**, 486–495.