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Immunology

Oxidative inactivation of leukotriene C_4 by stimulated human polymorphonuclear leukocytes

(neutrophils/oxidative metabolism)

CHONG W. LEE*, ROBERT A. LEWIS*, E. J. COREY†, ALAN BARTON†, HUNSEUNG OH†, ALFRED I. TAUBER*, AND K. FRANK AUSTEN*‡

*Department of Medicine, Harvard Medical School, and Department of Rheumatology and Immunology, Brigham and Women's Hospital, Boston, Massachusetts 02115; and †Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138

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ABSTRACT **Leukotriene C_4 (LTC_4) was metabolized by human polymorphonuclear leukocytes (PMNs) stimulated with phorbol myristate acetate (PMA) into three sets of products. These products differed in mobility on reverse-phase high-performance liquid chromatography (RP-HPLC) from LTC_4 and also from leukotriene D_4 (LTD_4) and leukotriene E_4 (LTE_4), the sequential products of peptide cleavage of LTC_4. Products I, II, and III were eluted as doublets with an average retention time for each doublet of 7.5 ± 0.3, 10.5 ± 0.6, and 16.3 ± 1.1 min (mean ± SD), respectively, as compared with 13.8 min for LTC_4. Doublet I material was biologically inactive and showed <5% of the immunoreactivity of LTC_4, doublet II material had 1% of the spasmogenic activity of LTC_4 on the guinea pig ileum and was equally immunoreactive, and doublet III material was neither biologically active nor immunoreactive. When [14,15-^{3}H]LTC_4 and [^{35}S]LTC_4 were metabolized, all three doublet products retained the ^{3}H label, whereas only the doublet I and doublet II products retained the ^{35}S label. The UV absorbance spectra of the three sets of metabolites were as follows: doublet I, maximum at 280 nm with shoulders at about 270 and 290 nm; doublet II, maximum at 284.5 nm with shoulders at about 275 and 295 nm; and doublet III, maximum at 269 nm with shoulders at about 259 and 279 nm. The metabolism of LTC_4 to the three classes of functionally inactive products by stimulated PMNs was completely blocked by catalase and azide, indicating a requirement for H_2O_2 and myeloperoxidase. When hypochlorous acid (HOCl)—considered to be a natural product of the interaction of myeloperoxidase, H_2O_2, and chloride ion—was formed chemically and allowed to react with LTC_4, the resulting products were indistinguishable by UV and HPLC analyses from the doublet II and doublet III metabolites of LTC_4. The doublet II products were identified as the two diastereoisomeric sulfoxides of LTC_4 by comparison with synthetic reference compounds. The doublet III products were shown to be identical with synthetic samples of (5*S*,12*S*)- and (5*S*,12*R*)-6-*trans*-LTB_4. The formation of two diastereoisomeric LTC_4 sulfoxides and 6-*trans*-LTB_4 can be explained in terms of an *S*-chlorosulfonium ion as the initial reactive intermediate, which subsequently undergoes conversion to product II by hydrolysis and product III by carbocation formation.**

Oxidative metabolism of arachidonic acid initiated by 5-lipoxygenase proceeds through a hydroperoxide intermediate to the formation of an unstable epoxide, 5,6-oxido-7,9-*trans*-11,14,-*cis*-icosatetraenoic acid (leukotriene A_4, LTA_4), which in turn can be converted enzymatically to (5*S*,6*R*)-5,6-dihydroxy-6,14-*cis*-8,10-*trans*-icosatetraenoic acid (leukotriene B_4, LTB_4) (1–3). Alternatively, LTA_4 can be converted to a C_6-sulfidopeptide, (5*S*,6*R*)-5-hydroxy-6-*S*-glutathionyl-7,9-*trans*-11,14-*cis*-icosatetraenoic acid (leukotriene C_4, LTC_4) by enzymatically catalyzed coupling with glutathione through a glutathione *S*-transferase. Then (5*S*,6*R*)-5-hydroxy-6-*S*-cysteinylglycyl-7,9-*trans*-11,14-*cis*-icosatetraenoic acid (leukotriene D_4, LTD_4) and (5*S*,6*R*)-5-hydroxy-6-*S*-cysteinyl-7,9-*trans*-11,14-*cis*-icosatetraenoic acid (leukotriene E_4, LTE_4) are generated from LTC_4 by the sequential cleavage of glutamic acid and glycine from the C_6-sulfidopeptide side chain (4–7).

Human and rabbit polymorphonuclear leukocytes (PMNs) challenged with calcium ionophore A23187 produce LTB_4 as the predominant leukotriene product as demonstrated after resolution on reverse-phase high-performance liquid chromatography (RP-HPLC) and characterization by mass spectroscopy (3, 8). Of cells that produce LTC_4, zymosan-stimulated mouse macrophages, zymosan-stimulated mouse pulmonary interstitial macrophages, and A23187-stimulated mouse mastocytoma cells retain the C_6-sulfidopeptide leukotriene in this form (4, 9, 10), whereas A23187-stimulated rat basophilic leukemic cells, mouse pulmonary alveolar macrophages, and rat peritoneal mononuclear cells convert LTC_4 to LTD_4 and LTE_4 in a time-dependent fashion (7, 10–12). Because the sequential cleavage of the C_6-sulfidopeptide chain of LTC_4 to give LTD_4 and LTE_4 is associated with only modest changes in the biological functions of these compounds (13–15), this pathway does not represent a metabolic inactivation scheme.

The present study shows that human PMNs stimulated with phorbol myristate acetate (PMA) metabolize LTC_4 to a mixture of biologically less active and inactive compounds by an oxidative pathway involving H_2O_2 and myeloperoxidase, in which hypochlorous acid, HOCl, appears to be an ultimate effector species.

MATERIALS AND METHODS

Materials. Hanks' balanced salt solution (HBSS) (Microbiological Associates, Bethesda, MD); Ficoll/Hypaque and macromolecular dextran (Pharmacia); atropine sulfate, histamine diphosphate, PMA, catalase (thymol free from bovine liver, 11,000 units/mg), sodium benzoate, superoxide dismutase (from bovine blood, 2,900 units/mg), glycine, and L-serine (Sigma); sodium azide (Fisher); and HPLC-grade methanol and acetonitrile (Burdick and Jackson Laboratories, Muskegon, MI) were purchased from the manufacturers. LTB_4, LTC_4, LTD_4, and LTE_4 were prepared and stored in 0.1 M phosphate buffer (pH 6.8) containing 20% (vol/vol) ethanol as described (13, 16, 17); before use, the leukotrienes were evaporated to dryness under a steady stream of nitrogen and resuspended in HBSS to give a stock solution of 25 μg/ml. [14,15-^{3}H]LTC_4 (60 Ci/mmol; 1 Ci = 3.7×10^{10} becquerels) and [^{35}S]LTC_4 (42 Ci/mmol) were obtained from New England Nuclear. HOCl was

Abbreviations: HBSS, Hanks' balanced salt solution; LTA_4, leukotriene A_4; LTB_4, leukotriene B_4; LTC_4, leukotriene C_4; LTD_4, leukotriene D_4; LTE_4, leukotriene E_4; PMA, phorbol myristate acetate; PMNs, polymorphonuclear leukocytes; RP-HPLC, reverse-phase high-performance liquid chromatography.

‡To whom reprint requests should be addressed.

vacuum-distilled from NaOCl (Chlorox) that had been adjusted to pH 7.5 with dilute sulfuric acid (18); the concentration was determined from A_{235} by assuming an extinction coefficient of 100 M^{-1} cm^{-1} (19). The concentration of H_2O_2 was calculated from A_{235} by using an extinction coefficient of 81 M^{-1} cm^{-1} (20).

Metabolism of LTC_4 by Human PMNs. Four to 10×10^8 PMNs were isolated from 500 ml of citrate-anticoagulated blood from normal donors to a purity of >97% by sequential dextran sedimentation, hypotonic lysis, and Ficoll/Hypaque gradient centrifugation (21); they were washed three times and resuspended in HBSS. Duplicate samples of 10^7 PMNs each were prewarmed and incubated with 3 μg of LTC_4 with and without 1 μg of PMA in HBSS (final volume, 1.05 ml) for various time intervals at 37°C. The reactions were stopped by centrifugation of the mixtures at 8,000 × *g* for 1 min at 4°C, and the supernatants were resolved by RP-HPLC for identification of LTC_4 metabolites. In additional experiments, the same concentrations of PMNs and PMA were first incubated for 30 min at 37°C to obtain the supernatants, which were then incubated with 3 μg of LTC_4 at 37°C for various time intervals before analysis by RP-HPLC.

For initial characterization of LTC_4 metabolites, RP-HPLC was carried out on a C_{18} (10 μm) Lichrosorb column (Altex Rainin, Berkeley, CA) at a flow rate of 1 ml/min in an isocratic solvent system of methanol/water/acetic acid, 65:34.9:0.1 (vol/vol), at pH 5.6, and sequential 0.5-ml fractions were collected. A_{280} of the column effluent was continuously monitored with an on-line model 100-40 spectrophotometer (Hitachi, Tokyo), and A_{280} peaks were calculated with a ChromatoPac R-1A data processor (Shimadzu, Kyoto, Japan). The column was calibrated for the retention times of LTB_4, LTC_4, LTD_4, and LTE_4, which were 21.8 ± 2.5, 13.8 ± 0.9, 21.8 ± 2.1, and 25.7 ± 2.1 min (mean ± SD, n = 4), respectively; A_{280} recoveries were 80.9 ± 3.6, 81.3 ± 10.8, and 81.1 ± 1.6% (mean ± SD, n = 3) for LTC_4, LTD_4, and LTE_4, respectively.

Characterization of Metabolic Products of LTC_4. Fractions absorbing at 280 nm with similar retention times were pooled from four experiments performed with neutrophils from different donors. The pooled fractions were evaporated to dryness and resuspended in HPLC buffer for determination of each UV absorbance spectrum on a model 552 spectrophotometer (Perkin–Elmer, Oak Brooks, IL). The pooled fractions then were reevaporated to dryness and resuspended in HBSS for assessment of spasmogenic and immunogenic activity. Spasmogenic activities were determined on the isolated guinea pig ileum suspended in Tyrode's buffer containing 1 μM atropine. One SRS unit of activity was defined as that amount giving a contraction equal in amplitude to that elicited by 5 ng of histamine (22).

For radioimmunoassay, net radioligand binding-inhibition curves were determined for standard dilutions of LTC_4 and for RP-HPLC-purified LTC_4 metabolites, with [^{3}H]LTC_4 as the radioligand and C_6-sulfidopeptide-specific immune rabbit plasma, as described (23). Unlabeled synthetic LTC_4 was detectable on the linear portion of the net radioligand binding-inhibition curves over a dose range of 0.1–5.0 ng.

RESULTS

Metabolism of LTC_4 by PMA-Stimulated PMNs. When 10^7 PMNs were incubated with 3 μg of LTC_4 in 1 ml HBSS at 37°C for 30 min and the supernatant was chromatographed on RP-HPLC, 84.0 ± 9.7% (mean ± SD, n = 4) of A_{280}-absorbing material was eluted in the position of LTC_4; the remainder was eluted predominantly as LTE_4 with small amounts of LTD_4. In contrast, the addition of 1 μg of PMA to parallel incubation mixtures resulted in a complete loss of A_{280} at the RP-HPLC retention time corresponding to LTC_4 and in the appearance of new peaks that did not correspond to the known retention times of LTD_4 and LTE_4. Incubation of 3 μg of LTC_4 for 30 min at 37°C with the supernatant of 10^7 PMNs stimulated with PMA under the usual conditions converted <5% of the LTC_4.

Activation of PMNs with various concentrations of PMA for 30 min at 37°C resulted in dose-related metabolism of LTC_4 as assessed by a decrement in the 280 nm-absorbing peak eluted from RP-HPLC with the retention time of LTC_4. This metabolism, stimulated by 1, 10, 100, and 1,000 ng of PMA, averaged 5%, 41%, 98%, and 100%, respectively, in two experiments. The time course of LTC_4 metabolism at 37°C by 10^6 and 10^7 cells per ml stimulated with 1 μg of PMA revealed a more rapid and complete metabolism at the higher cell concentration (Fig. 1). Thus, complete metabolism of LTC_4 was related to the presence of PMNs and their concentration and to the concentration of PMA.

Initial Characterization of Metabolites of LTC_4. New peaks with 280-nm absorbance that appeared concurrently with the loss of LTC_4 were resolved by RP-HPLC into three doublets, designated I, II, and III in order of their elution, as depicted for a single experiment in Fig. 2. For 28 consecutive experiments, including eight from which materials were pooled for determination of UV absorbance spectra, the retention times were 7.5 ± 0.3, 13.8 ± 0.9, and 16.3 ± 1.1 min (mean ± SD) for doublets I, II and III, respectively.

Duplicate samples of [14,15-^{3}H]LTC_4 (100,000 cpm) and 3 μg of unlabeled LTC_4 were incubated with 10^7 PMNs and 1 μg of PMA for 30 min either at 37°C or 0°C to determine whether any other metabolites were generated in addition to those absorbing at 280 nm and to establish quantitative recoveries from RP-HPLC. The usual isocratic elution of 30-min duration was followed by the application of a linear gradient of 65–100% methanol over 10 min and the subsequent elution with 100% methanol for an additional 20 min. The total recovery of radioactivity from the three peaks was 43,870 cpm, which was comparable to the recovery of 48,540 cpm from a single peak of radioactivity eluted at the retention time of LTC_4 after chromatography of the supernatant from the 0°C incubation mixture. The distribution of the ^{3}H label in products I, II, and III was 13%, 37%, and 50%, respectively.

Duplicate samples of [^{35}S]LTC_4 (500,000 cpm) and 3 μg of unlabeled LTC_4 were incubated with 10^7 PMNs and 1 μg of PMA for 30 min either at 37°C or 0°C to determine whether any of the metabolites contained sulfur. After the 37°C incubation,

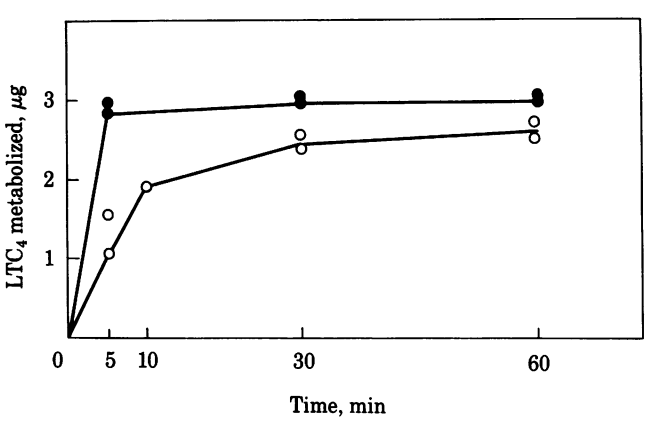

FIG. 1. Kinetics of metabolism of 3 μg of LTC_4 by 10^7 (●——●) and 10^6 (○——○) PMNs stimulated with 1 μg of PMA at 37°C. Metabolism of LTC_4 was quantitated by the disappearance of material absorbing at 280 nm and eluting at the retention time of LTC_4 after RP-HPLC of supernatants.

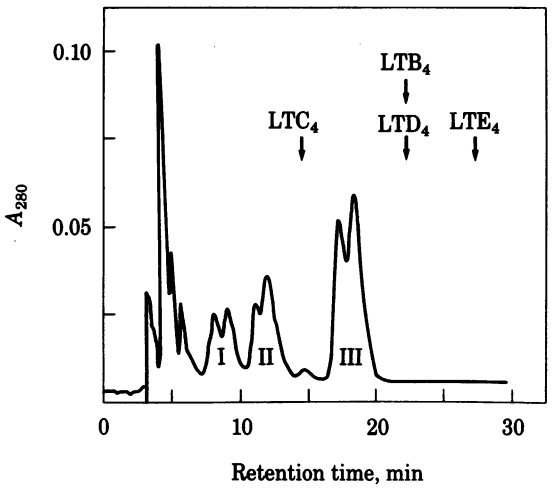

FIG. 2. RP-HPLC of a cell-free supernatant after incubation of 3 μg of LTC_4 with 10^7 PMNs and 1 μg of PMA for 30 min at 37°C. Retention times for synthetic LTB_4, LTC_4, LTD_4, and LTE_4 markers are indicated.

two peaks containing 5.0% and 9.4% of the recovered radioactivity, respectively, were eluted at the retention times of doublets I and II, respectively, whereas after the 0°C incubation, only a single peak, which contained 33% of the recovered radioactivity, was eluted at the retention time of LTC_4. Under both reaction conditions, the majority of the radioactive material was eluted at the front of the chromatogram as was the case for the starting material.

The UV absorbance spectrum of doublet I showed a maximum (λ_{max}) at 280 nm with shoulders at about 270 and 290 nm characteristic of natural C_6-sulfidopeptide leukotrienes, whereas the spectrum of doublet II was shifted bathochromically by 4.5 nm. Doublet III showed a maximum at 269 nm with shoulders at about 259 and 279 nm, corresponding more nearly to an LTB_4-type structure. Concentrations of the doublet I and II products were calculated from A_{280} by use of the molecular weight (625) and the extinction coefficient (40,000 cm^{-1} M^{-1}) of LTC_4. Concentration of the doublet III product was calculated from the absorbance at its λ_{max} by use of the molecular weight (336) and extinction coefficient (50,000 cm^{-1} M^{-1}) of LTB_4. Based upon these estimated concentrations, the specific spasmogenic activities on the guinea pig ileum of products characterized by doublets I, II, and III were assessed with up to 100 pmol of each. The components of doublets I and III were inactive at the highest amounts tested, whereas, for the doublet II product, the specific activity was 0.019 and 0.025 units/pmol in two experiments as compared to 1.4 units/pmol for synthetic LTC_4 (13).

In an experiment in which the amount of LTC_4 required for 50% inhibition of homologous radioligand binding by the class-specific anti-C_6-sulfidopeptide leukotriene immune plasma was 380 pg, comparable inhibition was achieved with 16,000 pg of doublet I, 420 pg of doublet II, and 10,000 pg of doublet III products. The finding that the doublet II product was comparable to LTC_4, whereas the doublet I and III products were <4% as immunoreactive, was also observed in two additional radioimmunoassay analyses.

Mechanisms of LTC_4 Metabolism by PMA-Stimulated PMNs. As PMA is known to trigger the respiratory burst of human PMNs (24) with generation of several oxygen species (25, 26), effects of these reactive oxygen intermediates on LTC_4 were examined by introducing agents known to remove or degrade them. Neither superoxide dismutase, which catalyzes the conversion of superoxide anion to H_2O_2 and O_2, nor dimethylsulfoxide, sodium benzoate, or ethanol, which scavenge hydroxyl radicals (27), had any effect on LTC_4 metabolism by PMA-stimulated PMNs (Table 1). However, catalase, which converts H_2O_2 to H_2O and O_2, and azide, which inhibits the activity of myeloperoxidase (28), each completely blocked the metabolism of LTC_4 to the three doublets by PMA-stimulated PMNs. L-Serine and glycine, scavengers of HOCl (29), blocked the metabolism of LTC_4 by 62% and 68%, respectively, at 20 mM. Inhibition by each of these reagents was not due to cytotoxicity because release of lactate dehydrogenase, 5.3 ± 1.2% (mean ± SD), was the same for PMA-stimulated PMNs in the absence of the inhibitors.

In order to define the nature of the oxygen species metabolizing LTC_4, 3 μg of LTC_4 was incubated in 1 ml of 10 μM H_2O_2 or 5 μM HOCl for 2 min at 37°C. After resolution by RP-HPLC, all of the H_2O_2-treated LTC_4 was eluted with the retention time of the original material. The reaction of HOCl with LTC_4 yielded as major products four compounds corresponding by RP-HPLC analysis to those comprising doublets II and III from PMA-stimulated PMNs.

Identification of Doublet-II Metabolites of LTC_4. Incubation of LTC_4 (500 μg) in 2 ml of 0.05 M phosphate buffer (pH 7.0) containing 20% ethanol with sodium periodate (3 mg, about 20 equivalents) for 1 hr at 23°C resulted in efficient conversion to a mixture of diastereoisomeric LTC_4 sulfoxides (30) with only a small percentage of LTC_4 remaining as assessed by RP-HPLC. The two LTC_4 sulfoxides were separated by an additional RP-HPLC with a C_{18} μ-Bondapak column (Waters Associates) and an isocratic solvent [acetonitrile/water/acetic acid, 34.9:65:0.1 (vol/vol)] at pH 5.6 and a flow rate of 0.5 ml/min; the two LTC_4 sulfoxides had retention times of 11.0 and 11.7 min, as compared to 17.4 min for LTC_4, and a total yield of 87%. Both sulfoxides showed A_{max} at about 284.5 nm with shoulders at 275 and 295 nm.

Each of the synthetic sulfoxides corresponded to one of the components of doublet II with regard to RP-HPLC behavior in both the methanol/water and acetonitrile/water systems. Further, the UV absorbance maxima were the same (284.5 ± 0.5 nm, mean ± SEM) within experimental error.

The same two diastereoisomeric LTC_4 sulfoxides were obtained by reaction of LTC_4 and HOCl at 23°C in aqueous methanol at pH 6.8 with *N*-chlorosuccinimide as a source of HOCl

Table 1. Effects of inhibitors of oxidative metabolism on LTC_4 metabolism by stimulated PMNs*

Inhibitors	Concentration		LTC_4 metabolized, %
	units/ml	mM	
Superoxide dismutase	200		99.4
	1,000		99.3
Catalase	200		16.5
	1,000		0
	1,000†		100
Dimethyl sulfoxide		1.0	100
Ethanol		1.0	100
Sodium benzoate		10.0	100
NaN_3		0.1	0
L-Serine		20.0	38
Glycine		20.0	32

* Quantitative disappearance of A_{280} corresponding to the retention time of LTC_4 was used to determine metabolism at 37°C. The recovery of LTC_4 from mixtures incubated at 0°C was 88 ± 6% (mean ± SD) of the starting material and was defined as 0% metabolism for each experiment. The results shown are the average of two experiments.

† Heat inactivated.

or, alternatively, at pH 6.0 with calcium hypochlorite and sulfonic ion-exchange resin in water used as the source of HOCl.

Identification of Doublet III Metabolites of LTC_4. Because the RP-HPLC and UV absorption data for the components of doublet III corresponded closely to those previously observed for the (12*R*)- and (12*S*)-diastereoisomers of 6-*trans*-LTB_4 (31), a comparison was made between doublet III material and samples of totally synthetic (12*S*)- and (12*R*)-6-*trans*-LTB_4. UV absorbance spectra of methanol solutions were indistinguishable, with the maximum (center peak) at 268.5 ± 0.5 nm and shoulders at about 259 and 279 nm for both synthetic and doublet III samples. RP-HPLC comparison on a C_{18} column (3 μm; Altex Rainin) with methanol/water/acetic acid, 75:24.9:0.1 (vol/vol), at pH 5.6 and a flow rate of 3 ml/min showed that the faster moving component of doublet III corresponded in retention time to (5*S*,12*R*)-6-*trans*-LTB_4 (11.2 min), whereas the slower-moving component corresponded to (5*S*,12*S*)-6-*trans*-LTB_4 (12.2 min). Correspondence was also ascertained by the exact superimposition of RP-HPLC peaks when synthetic compounds and the metabolic products were coinjected. The same findings were obtained with methanol/water/acetic acid, 65:34.9:0.1 (vol/vol), at pH 5.6.

The components of the doublet III product were esterified by using diazomethane in ether/methanol and compared with synthetic samples by RP-HPLC on the C_{18} μ-Bondapak column at a flow rate of 2 ml/min. The retention times of the methyl esters derived from doublet III material corresponded exactly to those of the methyl esters of synthetic (5*S*,12*R*)-6-*trans*-LTB_4 (6.6 min) and (5*S*,12*S*)-6-*trans*-LTB_4 (7.4 min) in methanol/water/acetic acid, 74:25.9:0.1 (vol/vol), at pH 5.6.

Mass spectral comparisons of the bistrimethylsilyl ethers of the doublet III methyl esters and the synthetic 6-*trans*-LTB_4 methyl esters were performed with a Kratos MS-50 spectrophotometer with a direct insertion probe at 250°C, accelerating voltage of 8 kV, and ionizing voltage of 70 eV (32). Ions were observed with both doublet III and synthetic samples at *m/e* 494 (M^+), 463, 383, 293, and 217, consistent with the formulation of the doublet III components as the (12*R*)- and (12*S*)-diastereoisomers of 6-*trans*-LTB_4.

DISCUSSION

The metabolic inactivation of LTC_4 by isolated human PMNs activated with PMA was accompanied by the generation of three pairs of products. As assessed by recovery of synthetic LTC_4 after RP-HPLC, its metabolic inactivation was dependent on the concentration of PMA, the duration of interaction between LTC_4 and the cells, and the concentrations of the PMNs. Under conditions at 37°C in which 10^7 cells stimulated with 1 μg of PMA inactivated all of 3 μg of synthetic LTC_4 in the first 5 min, 85% of the initial LTC_4 remained after a 30-min incubation with unstimulated PMNs (Fig. 1). Further, the supernatant harvested after a 30-min incubation of PMA-activated PMNs did not inactivate LTC_4 during a further 30-min incubation at 37°C, indicating that the mechanism for metabolic inactivation of LTC_4 was dependent upon the presence of a labile effector.

Metabolic conversion products of unlabeled synthetic LTC_4 and [^{3}H]LTC_4, resolved with the same retention times on RP-HPLC, were designated I, II, and III in their elution order (Fig. 2); there were no additional ^{3}H-labeled products. The doublet I product showed the UV absorbance spectrum of a C_6-sulfidopeptide leukotriene, with λ_{max} at 280 nm and shoulders at about 270 and 290 nm, but showed <4% of the immunoreactivity of LTC_4 with class-specific immune plasma and <1% of the spasmogenic activity of LTC_4 on the guinea pig ileum. Because doublet I constituted only a small percentage of the total products, chemical identification was deferred until large quantities could be accumulated. The doublet II product had a λ_{max} at 284.5 nm with shoulders at about 275 and 295 nm, showed immunoreactivity comparable to that of LTC_4, and had 1% of the spasmogenic activity of LTC_4. This material cochromatographed with the two sulfur diastereoisomers of LTC_4 sulfoxide. The doublet III product had a λ_{max} at 269 nm with shoulders at about 259 and 279 nm, exhibited <4% of the immunoreactivity of LTC_4, and had <1% of the spasmogenic activity of LTC_4. The first peak of doublet III corresponded to (5*S*,12*R*)-6-*trans*-LTB_4 and the second peak of doublet III corresponded to (5*S*,12*S*)-6-*trans*-LTB_4 as shown by RP-HPLC comparisons both separately and by using cochromatography. Identity was further demonstrated by RP-HPLC comparison of methyl esters and mass spectral comparison of trimethylsilylated methyl esters.

Because the concentration of PMA recognized as optimal for metabolic inactivation of LTC_4 was appropriate for eliciting the respiratory burst (24) and was higher than that required for eliciting secretory granule release (33), it seemed likely that the metabolism was oxidative. This possibility was supported by the identification of the doublet II product as being comprised of the two chiral sulfoxides of LTC_4. The failure of superoxide dismutase and of the hydroxyl radical scavengers benzoate, ethanol, and dimethyl sulfoxide to prevent the inactivation of LTC_4 and the appearance of doublets I, II, and III indicated that neither superoxide anions nor hydroxyl radicals are involved in the oxidative metabolism of LTC_4. The ability of catalase to prevent the inactivation of LTC_4 indicated the importance of H_2O_2 formation in the appearance of metabolic products. The finding that azide also prevented LTC_4 metabolism indicated that the action of myeloperoxidase on H_2O_2 is a necessary step in the formation of oxidizing species. That the critical species product by stimulated PMNs may be HOCl was supported by the ability of L-serine and glycine, scavengers of

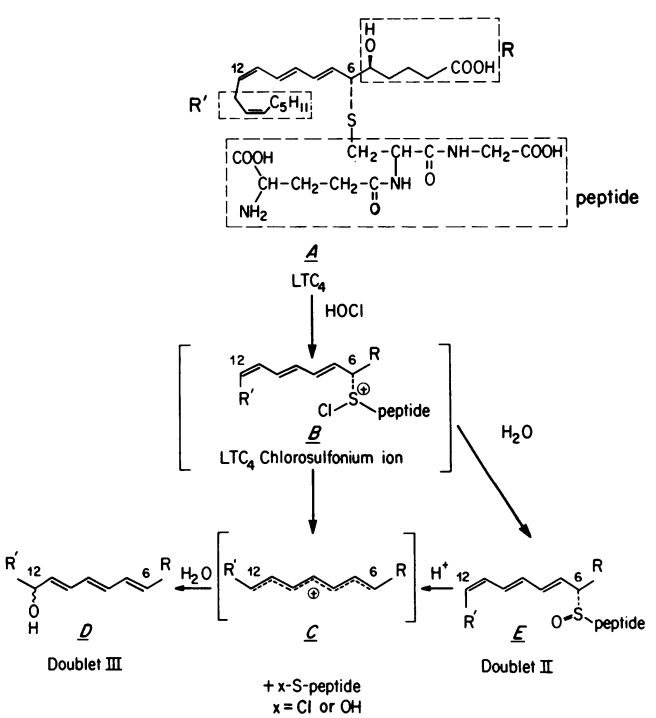

FIG. 3. Proposed chemical mechanism for myeloperoxidase-dependent metabolism of LTC_4. R = —$CHOH(CH_2)_3CO_2H$; S-peptide = glutathione; R′ = —$CH_2CH{=}CHC_5H_{11}$.

HOCl, to block the metabolic inactivation of LTC_4.

The chemical interaction of HOCl and synthetic LTC_4 for 2 min at 37°C, followed by RP-HPLC resolution of the products, duplicated the actions of PMA-stimulated PMNs in forming doublets II and III. This chemical conversion of LTC_4 to a mixture of two diastereoisomeric LTC_4 sulfoxides and (12*R*)- and (12*S*)-diastereoisomers of 6-*trans*-LTB_4 can be accommodated by the mechanistic pathways outlined in Fig. 3. Reaction of LTC_4 (Fig. 3*A*) with HOCl is expected to generate initially the S-chlorosulfonium ion (Fig. 3*B*) as a mixture of diastereoisomers (34). This species can undergo facile heterolysis of the C—S bond to form the highly stabilized heptatrienyl cation system (Fig. 3*C*), which, upon reaction with water, is expected to afford the (12*R*)- and (12*S*)-diastereoisomers of 6-*trans*-LTB_4 (Fig. 3*D*) as observed in the acid-catalyzed hydrolysis of LTA_4 (32). Alternatively, nucleophilic attack on the diastereoisomeric S-chlorosulfonium ion (Fig. 3*B*) at sulfur by water or hydroxide ion would produce the diastereoisomeric LTC_4 sulfoxides (Fig. 3*E*) (34). An alternative pathway to the diastereoisomeric 6-*trans*-LTB_4 compounds involves the formation of the heptatrienyl cation (Fig. 3*C*) from the sulfoxide (Fig. 3*E*) by [3,2]-sigmatropic rearrangement (34) and acid catalysis through an S-hydroxysulfonium ion. The rate of this process is relatively slow and its contribution is only minor.

The ability of human PMNs to metabolize LTC_4 to biologically inactive products when engaged in the respiratory burst contrasts with the capacity of the same cell when activated with calcium or a smaller dose of PMA to release a LTD_4 dipeptidase, from the specific granule, that converts LTD_4 to LTE_4 (35). The finding that the same cell can process exogenous leukotrienes differently, depending upon the nature and intensity of the stimulus, has important implications for quantitatively assessing the biosynthetic capacity of such cells in response to various stimuli. Presumably, agonists evoking a minimal respiratory burst, concomitant to initiating release of arachidonic acid from membrane phospholipids, would be most favorable for the generation of peptidoleukotrienes for the cells with a 5-lipoxygenase. The respiratory burst appears to be a general control mechanism for inactivating diverse biological products (36, 37).

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