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Leukotriene A_4 hydrolase: Protection from mechanism-based inactivation by mutation of tyrosine-378

(suicide inactivation/inflammation)

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ABSTRACT **Leukotriene A_4 (LTA_4) hydrolase [(7*E*,9*E*, 11*Z*,14*Z*)-(5*S*,6*S*)-5,6-epoxyicosa-7,9,11,14-tetraenoate hydrolase; EC 3.3.2.6] is a bifunctional zinc metalloenzyme that catalyzes the final step in the biosynthesis of the potent chemotactic agent leukotriene B_4 (LTB_4). LTA_4 hydrolase/aminopeptidase is suicide inactivated during catalysis via an apparently mechanism-based irreversible binding of LTA_4 to the protein in a 1:1 stoichiometry. Previously, we have identified a henicosapeptide, encompassing residues Leu-365 to Lys-385 in human LTA_4 hydrolase, which contains a site involved in the covalent binding of LTA_4 to the native enzyme. To investigate the role of Tyr-378, a potential candidate for this binding site, we exchanged Tyr for Phe or Gln in two separate mutants. In addition, each of two adjacent and potentially reactive residues, Ser-379 and Ser-380, were exchanged for Ala. The mutated enzymes were expressed as $(His)_6$-tagged fusion proteins in *Escherichia coli*, purified to apparent homogeneity, and characterized. Enzyme activity determinations and differential peptide mapping, before and after repeated exposure to LTA_4, revealed that wild-type enzyme and the mutants [S379A] and [S380A]LTA_4 hydrolase were equally susceptible to suicide inactivation whereas the mutants in position 378 were no longer inactivated or covalently modified by LTA_4. Furthermore, in [Y378F]LTA_4 hydrolase, the value of k_{cat} for epoxide hydrolysis was increased 2.5-fold over that of the wild-type enzyme. Thus, by a single-point mutation in LTA_4 hydrolase, catalysis and covalent modification/inactivation have been dissociated, yielding an enzyme with increased turnover and resistance to mechanism-based inactivation.**

Leukotriene A_4 (LTA_4) hydrolase (EC 3.3.2.6.) is a bifunctional zinc metalloenzyme which converts LTA_4 into the proinflammatory substance LTB_4, a reaction referred to as the epoxide hydrolase activity (1). In addition, the enzyme possesses an aminopeptidase activity whose endogenous substrate and physiological significance are unknown (2–4).

Previous studies with site-directed mutagenesis have identified His-295, His-299, and Glu-318 as the three zinc-binding ligands (5). With the same technique, Glu-296 was shown to be a catalytic amino acid for the peptidase activity, a finding which allowed us to propose a general base mechanism for the peptide hydrolysis (6). Since the epoxide hydrolase activity was not affected by mutation of Glu-296, the data also showed that the active site(s), corresponding to the two enzyme activities, are not identical although they share several structural and functional properties. Furthermore, sequence comparisons with aminopeptidase M has pointed to Tyr-383 as another possible catalytic amino acid and subsequent mutational analysis has revealed that Tyr-383 is essential for the peptidase reaction of LTA_4 hydrolase, where it may act as a proton donor (7). Moreover, experiments with chemical modification have indicated that tyrosyl and arginyl residues are catalytically important for both the epoxide hydrolase and the peptidase activity (8). However, with the exception of the catalytic zinc, no specific functional elements involved in the epoxide hydrolysis have yet been identified.

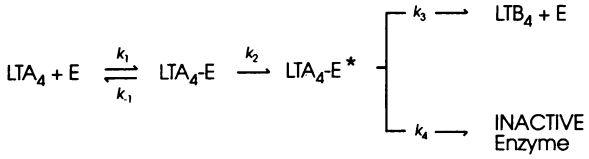

FIG. 1. Schematic representation of mechanism-based inactivation. LTA_4-E* represents the activated enzyme-substrate complex that can either break down to free enzyme and product or result in covalent bond formation and inactivation. The partition ratio is determined by the rate constants k_3 and k_4 (12).

During catalysis, LTA_4 hydrolase is covalently modified and inactivated by its natural lipid substrate LTA_4, a process commonly referred to as suicide inactivation (9, 10). LTA_4 hydrolase has been proposed to be the rate-limiting enzyme in cellular LTB_4 biosynthesis and suicide inactivation may therefore be an important mechanism for the overall regulation of this biosynthetic pathway *in vivo* (11). Suicide inactivation of LTA_4 hydrolase satisfies several criteria of a mechanism-based process (Fig. 1) (12, 13). For instance, after treatment of the enzyme with LTA_4 or LTA_4 methyl ester, the epoxide hydrolase and peptidase activities are lost simultaneously and irreversibly in a time-dependent, saturable process that is of pseudo first-order kinetics and dependent upon catalysis. Active-site specificity has been demonstrated by protection with competitive inhibitors (13, 14) and mass spectrometric analysis has revealed that suicide inactivation occurs predominantly in a 1:1 stoichiometry between lipid and protein, with only little modification of secondary sites (13).

Recently, we have used differential peptide mapping of unmodified and suicide inactivated LTA_4 hydrolase to identify a peptide segment, encompassing residues 365–385, involved in the binding of LTA_4 and LTA_4 methyl and ethyl esters to the native enzyme (15). Amino acid sequence analysis of a modified form of this peptide, isolated from a digest of enzyme inactivated with LTA_4 ethyl ester, indicated that Tyr-378 could be responsible for the LTA_4 binding. In the present study, we have employed site-directed mutagenesis to detail the importance of Tyr-378 for catalyses and mechanism-based inactivation. We show that a mutagenetic replacement of Tyr-378 that removes its hydroxyl group increases the turnover for epoxide

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Abbreviations: LTA_4 and LTB_4, leukotrienes A_4 and B_4.

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hydrolysis and makes the enzyme virtually resistant to inactivation/covalent modification with LTA_4.

EXPERIMENTAL PROCEDURES

Materials. LTA_4 ethyl ester (Merck–Frosst Labs, Pointe Claire, PQ Canada) was saponified in tetrahydrofuran with 1 M LiOH (6% vol/vol) for 48 h at 4°C. Alanine-4-nitroanilide, sodium iodoacetate, and DTT were from Sigma. T7 sequencing kit, restriction endonucleases, T4 DNA ligase, and PD-10 columns were purchased from Pharmacia and nickel-NTA resin was from Qiagen (Chatsworth, CA). Oligonucleotides were synthesized by Scandinavian Gene Synthesis. Lys-C protease (150 units/mg, Boehringer Mannheim) was dissolved in 1 mM HCl and stored up to 4 weeks at −20°C.

PCR Mutagenesis of Human Recombinant LTA_4 Hydrolase cDNA. A cDNA fragment (1907 bp), containing the entire coding sequence of human LTA_4 hydrolase, was cloned into the *Escherichia coli* expression vector pT3–5LO, constructed from the phagemid pT7T3 (16), to produce pT3-MB_3. Six histidine codons were introduced immediately after the initiation codon by PCR mutagenesis using a synthetic oligonucleotide (5′-GAT AAC GAA TTC ATG CAT CAC CAC CAT CAC CAT CCC GAG ATA GTG GAT ACC TGT TC-3′). This upstream primer contains an *Eco*RI restriction site before the histidine codons, and the downstream primer used covers a unique *Bgl*II site. The amplified PCR fragment (digested with *Eco*RI and *Bgl*II) was ligated together with the remaining 3′ end of the LTA_4 hydrolase cDNA (cleaved with *Bgl*II and *Pst*I) into pT3-MB_3 (opened with *Eco*RI and *Pst*I) to produce the expression plasmid pT3-MB_4.

Site-directed mutagenesis of the human LTA_4 hydrolase cDNA in pT3-MB_4 was carried out by PCR according to the "megaprimer" method (17). In a first PCR reaction a "megaprimer" was amplified by using primer A containing the mutation and primer B covering a unique *Bfr*I site (Table 1). The megaprimer was used in a second PCR reaction, together with primer C covering a unique *Bgl*II site (Fig. 2). After cleavage of the PCR fragment with *Bgl*II/*Bfr*I it was ligated into the pT3-MB_4 plasmid that had been previously cut with the same restriction enzymes. The entire cDNA of each mutated plasmid was sequenced by the dideoxy chain-termination method (18) to verify the respective mutation and to confirm that no other changes of the nucleotide sequence had occurred.

Affinity Chromatography. The recombinant proteins were expressed in *E. coli* (JM101) cells essentially as described (7). The cells were cultured in M9 medium at 37°C and after induction with isopropyl-β-D-galactopyranoside the incubation was continued for 4–5 h. Following centrifugation (1000 × *g* for 10 min), the cells were resuspended in homogenization buffer (50 mM sodium phosphate, pH 8.0/300 mM NaCl/0.2 μg/ml soybean trypsin inhibitor/5 mM 2-mercaptoethanol) and sonicated for 3 × 10 s on ice with a Branson S-125 sonicator (setting 4). Following an additional centrifugation (15,000 × *g* for 10 min), the supernatant was filtered through a 0.2-μm filter and applied to a column containing a nickel-NTA (nitrilo triacetic acid) resin. The column was washed with

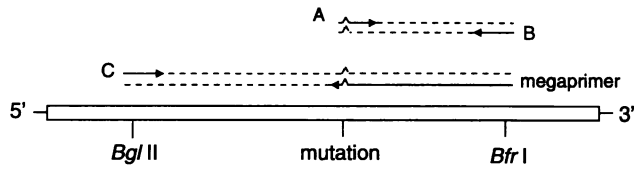

FIG. 2. PCR mutagenesis of human LTA_4 hydrolase cDNA. Primers A and B, the former carrying the point mutation(s), were used in a first round of PCR to generate a megaprimer. This megaprimer was in turn used in a second PCR reaction, together with primer C, to generate a mutated fragment, which after digestion with *Bgl*II and *Bfr*I could be ligated into the expression plasmid. The open box represents only a part of the cDNA.

2 bed volumes of 100 mM Tris·HCl (pH 8.0), followed by 5 bed volumes of 1 M NaCl in 50 mM sodium phosphate (pH 6.0) and 2 bed volumes of 100 mM Tris·HCl (pH 8.0). Finally, the protein was eluted with 2 bed volumes of 50 mM Tris·HCl (pH 8.0), containing 100 mM imidazole. After gel filtration on a PD-10 column, the protein was applied to a hydroxyapatite column (TSKgel HA-1000, 7.5 cm × 7.5 mm; TosoHaas, Montgomery, PA) pre-equilibrated with 10 mM sodium phosphate buffer (pH 7), containing 200 μM of $CaCl_2$. After elution of nonadsorbed material, the protein was eluted with a linear gradient of phosphate by continuous mixing with 300 mM sodium phosphate (pH 7), supplemented with 10 μM $CaCl_2$, at a flow rate of 0.4 ml/min. Active fractions were pooled and the buffer exchanged to 10 mM Tris·HCl (pH 8). Protein concentrations were determined by UV using the equation: [protein] = (A_{280} × 1.4 − A_{260} × 0.7) mg/ml.

Enzyme Assays. Epoxide hydrolase activity was determined from incubations of 2 μg of LTA_4 hydrolase in 200 μl of 50 mM Tris·HCl (pH 8.0), with 2 μl of the substrate LTA_4 (2.4–2.8 nmol/μl) at room temperature. The reaction was stopped after 15 s by addition of 2 vol of methanol, and prostaglandin B_1 was added as internal standard. LTB_4 was extracted and quantitated by reversed-phase HPLC as described (19). The aminopeptidase activity was determined spectrophotometrically with 2 μg of purified enzyme in 50 mM Tris·HCl (pH 7.5), containing 100 mM NaCl and 38 μg/ml bovine serum albumin with 1 mM alanine-4-nitroanilide as substrate (19).

Inactivation Experiments and Peptide Mapping. To obtain LTA_4-treated enzyme for peptide mapping, LTA_4 hydrolase (2 ml, 100 μg/ml in 50 mM Tris·HCl, pH 8.0) was incubated five times with 14 μM of LTA_4 for 30 min at room temperature (final concentration 70 μM). A portion of the reaction mixture (100 μl) was then gel-filtered on a PD-10 column and the eluted protein was analyzed for residual epoxide hydrolase and peptidase activity. The remaining enzyme was subjected to Lys-C digestion and differential peptide mapping by reversed-phase HPLC, as described (15). Peptides K21 (15) and [Y378F]K21 were identified by amino acid sequence analysis. For separate tests of suicide inactivation, small-scale (100 μl) incubations of LTA_4 hydrolase (100 μg/ml in 50 mM Tris·HCl, pH 8.0) with 3 × 2.6 μM LTA_4 (final concentration 78 μM) were performed followed by gel filtration (PD-10) and activity determinations.

Table 1. Primers used for PCR mutagenesis of human LTA_4 hydrolase cDNA

Mutation	Type of primer	Nucleotide sequence (5′ → 3′)	Numbering
Tyr-378 → Phe-378	A	d(TAG CTT TTT CTT CAG TTC CCT ATG)	1130–1153
Tyr-378 → Gln-378	A	d(TAG CTC AAT CTT CAG TTC CCT ATG)	1130–1153
Ser-379 → Ala-379	A	d(CTT ATG CTT CAG TTC CCT ATG AG)	1133–1155
Ser-380 → Ala-380	A	d(ATT CTG CAG TTC CCT ATG AGA AG)	1136–1158
None	B	d(CTT TGA CTT AAG GCA ATA CAA GC)	1424–1402
None	C	d(GCA GAA GAT CTG GGA GGA CCG TAT G)	733–757

Mutated bases are underlined. Numbering, either forward or reverse, is according to the nucleotide sequence.

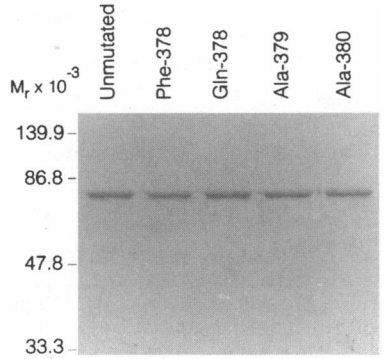

FIG. 3. SDS/PAGE of purified LTA_4 hydrolase mutants. Samples of 1 μg of the mutants [Y378F], [Y378Q], [S379A], and [S380A]LTA_4 hydrolase as well as wild-type enzyme were subjected to SDS/PAGE as described.

Circular Dichroism Spectrometry. Circular dichroism was performed with Aviv circular dichroism spectrometer model 62DS (Lakewood, NJ). Purified recombinant enzyme was analyzed in 10 mM sodium phosphate buffer (pH 7.5), at a concentration of 1 mg/ml.

Electrophoresis. SDS/PAGE was done in a Mini-Protean II dual slab cell (Bio-Rad). The purified recombinant proteins were boiled in Laemmli sample buffer [62.5 mM Tris·HCl (pH 6.8)/2% SDS/10% glycerol/5% 2-mercaptoethanol/0.001% bromphenol blue] and loaded on a polyacrylamide gel (10% separating gel/5% stacking gel). Bands of protein were stained with Coomassie brilliant blue.

RESULTS

Mutagenetic Replacements and Expression of Recombinant Enzymes. To study the role of Tyr-378 in catalyses and suicide inactivation, this residue was replaced by phenylalanine or glutamine and two adjacent serines at positions 379 and 380 were each replaced by alanine to produce the four separate mutants [Y378F], [Y378Q], [S379A], and [S380A]LTA_4 hydrolase. Wild-type and mutated human LTA_4 hydrolases were expressed in *E. coli* as fusion proteins containing six histidine residues at the N terminus allowing rapid purification on nickel-affinity chromatography in high yield (10 mg/liter cell culture). An additional step of hydroxyapatite chromatography resulted in apparently homogenous enzyme preparations (Fig. 3). The specific epoxide hydrolase and peptidase activities

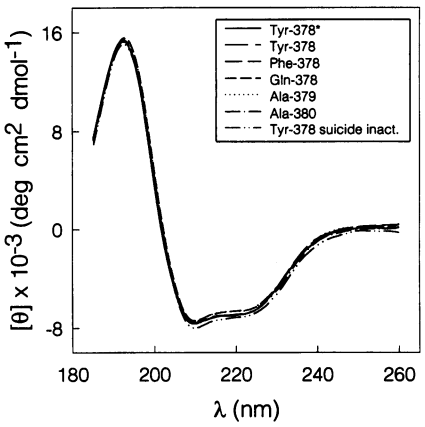

FIG. 4. Circular dichroism spectra of wild-type, suicide inactivated, or mutated LTA_4 hydrolase. The spectra were recorded in 10 mM of sodium phosphate (pH 7.5), and data are expressed as mean residue ellipticity, which is the molar ellipticity divided by the number of amino acid residues in the protein measured. Tyr-378* denotes wild-type enzyme lacking a $(His)_6$-tag.

Table 2. Specific epoxide hydrolase and peptidase activities of wild-type and mutated LTA_4 hydrolase

	Epoxide hydrolase activity		Peptidase activity	
Enzyme	nmol/mg/min	%	nmol/mg/min	%
Wild type	692 ± 6	100 ± 0.9	188 ± 8	100 ± 4
[Y378F]	1197 ± 23	173 ± 3	81 ± 4	43 ± 2
[Y378Q]	233 ± 7	34 ± 1	3 ± 0.3	1.6 ± 0.2
[S379A]	179 ± 8	26 ± 1	8 ± 0.1	4 ± 0.1
[S380A]	371 ± 7	54 ± 1	128 ± 7	68 ± 4

Results are mean ± SD; $n = 3$.

were similar for wild-type enzyme, with or without the His- tag. Furthermore, the peptide region of the circular dichroism profile of all the mutated enzymes exhibited no significant changes compared with the unmutated enzyme (with or without His-tag), suggesting that no major alterations of the tertiary structures had occurred (Fig. 4). No changes of the circular dichroism spectrum occurred upon 59% inactivation of wild-type enzyme with LTA_4 methyl ester, indicating that loss of enzyme activity, associated with covalent modification, is not due to major structural changes of the protein (Fig. 4).

Effects of Mutations on the Enzyme Activities. Although the effects of mutations on enzyme activities varied, all mutated enzymes displayed a significant epoxide hydrolase and peptidase activity, indicating that none of the exchanged amino acids is critical for catalyses (Table 2). Except for [Y378F]-LTA_4 hydrolase, which displayed an increased epoxide hydrolase activity (173% of the wild-type enzyme), all mutants exhibited somewhat reduced epoxide hydrolase activities ranging from 54–26%. The peptidase activities of mutants [Y378F] and [S380A] were 43 and 68% of control, respectively, whereas the activities of [Y378Q] and [S379A] were below 5% of the control.

Effects of Mutations on Mechanism-Based Inactivation. Substrate mediated irreversible inactivation was determined by incubation of the enzymes (10 μg in 100 μl 50 mM Tris·HCl, pH 8) with three consecutive additions of 1 μl 2.6 mM LTA_4 (final concentration 78 μM) followed by gel-filtration and subsequent analysis of the remaining enzyme activities (Fig. 5). After treatment with LTA_4, unmutated LTA_4 hydrolase and the mutants [S379A] and [S380A] lost approximately 50% of their epoxide hydrolase and peptidase activity. In contrast, the mutants of Tyr-378 were not susceptible to substrate-mediated inactivation and the remaining epoxide hydrolase activities of

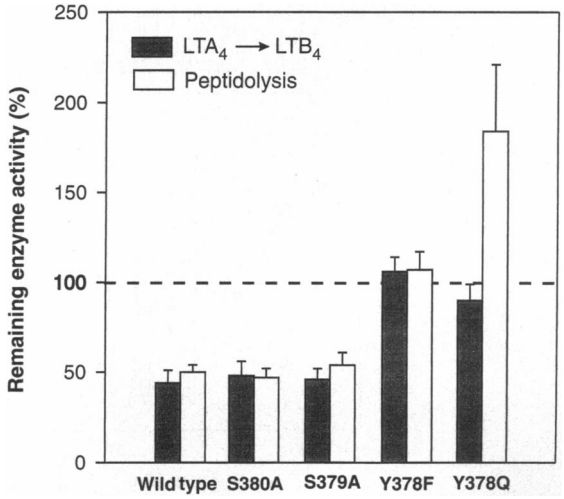

FIG. 5. Inactivation of LTA_4 hydrolase mutants by LTA_4. The histogram depicts remaining enzyme activities after three additions of 26 μM of LTA_4, expressed in percent of the respective untreated control. Each bar represents mean ± SD, $n = 3$.

Table 3. Kinetic constants for hydrolysis of LTA_4 and alanine-4-nitroanilide by wild-type and [Y378F]LTA_4 hydrolase

	Epoxide hydrolase activity		Peptidase activity	
	Wild type	[Y378F]	Wild type	[Y378F]
K_m, μM	5.8	23.1	470	3300
V_{max}, nmol·mg^{-1}·min^{-1}	735	1805	183.5	166.4
k_{cat}, s^{-1}	0.85	2.09	0.21	0.19
k_{cat}/K_m, $s^{-1}\cdot M^{-1}$	147×10^3	90.5×10^3	447	58.3

All values were obtained with $(His)_6$-tagged human recombinant enzymes.

[Y378F] and [Y378Q]LTA_4 hydrolase were 106 ± 8% and 90 ± 9%, respectively (Fig. 5). Similarly, the peptidase activities of these mutants remained unaffected after treatment with LTA_4, and in the case of [Y378Q] even a slight increase in catalytic activity was observed (184 ± 37%), which however still corresponded to < 5% of wild-type enzyme (see Table 2).

Kinetic Properties of [Y378F]LTA_4 Hydrolase. Mechanism-based inactivation of LTA_4 hydrolase has been shown to be turnover dependent (13). Therefore, the mutant [Y378F] was selected for further kinetic analysis. This enzyme displayed a somewhat higher K_m for LTA_4 (23.1 μM) than did the wild-type enzyme (K_m = 5.8 μM) but exhibited a higher turnover with a k_{cat} = 2.09 s^{-1} versus 0.85 s^{-1} for the unmutated control (Table 3). The mutation also reduced the binding of the synthetic substrate alanine-4-nitroanilide but had essentially no influence on the turnover of this amide (Table 3).

Peptide Mapping of Enzyme Treated with LTA_4. To determine the effects of mutation on covalent modification of LTA_4 hydrolase by LTA_4, large-scale inactivation experiments (200 μg enzyme) with subsequent proteolytic cleavage and HPLC peptide mapping were performed. As reported previously (15), both activities and a henicosapeptide, denoted K21, were lost in parallel during suicide inactivation of wild-type enzyme

Table 4. Suicide inactivation of wild-type LTA_4 hydrolase and mutants [Y378F] and [S380A] with LTA_4: Residual enzyme activities and amounts of unmodified peptides K21, [Y378F]K21, and [S380A]K21

Enzyme	Epoxide hydrolase activity, %	Peptidase activity, %	Unmodified peptide, %
Wild type	57	56	50
[Y378F]	99	101	104
[S380A]	39	35	46

Enzymes were treated with 5 × 14 μM LTA_4 prior to peptide mapping and activity determinations. The epoxide hydrolase activity, peptidase activity, and amount of peptides K21, [Y378F]K21, and [S380A]K21 (all spanning Leu-365 to Lys-385) in peptide maps of enzymes treated with LTA_4 are expressed in the percent of values obtained with corresponding untreated control enzymes.

(Table 4, Fig. 6). The mutant [S380A] was also analyzed by peptide mapping and was found to exhibit a similar correlation between degree of inactivation and modification of peptide [S380A]K21. In contrast, the epoxide hydrolase and peptidase activity of the enzyme [Y378F] as well as the recovery of the peptide [Y378F]K21 (positively identified by amino acid sequence analysis) were not affected by this treatment with LTA_4 (Table 4, Fig. 6). In agreement with previous findings (15), the loss of peptide K21 and [S380A]K21 in peptide maps of inactivated wild-type and [S380A]LTA_4 hydrolase was accompanied by the appearance of several more lipophilic peaks, presumably derived from modification of Tyr-378 in peptides K21 and [S380A]K21 (data not shown). The presence of more than one such peptide peak suggests that there may be other residues present in K21 with the ability to bind LTA_4. However, these lipophilic peptides were recovered in low yield and were not subjected to further analysis.

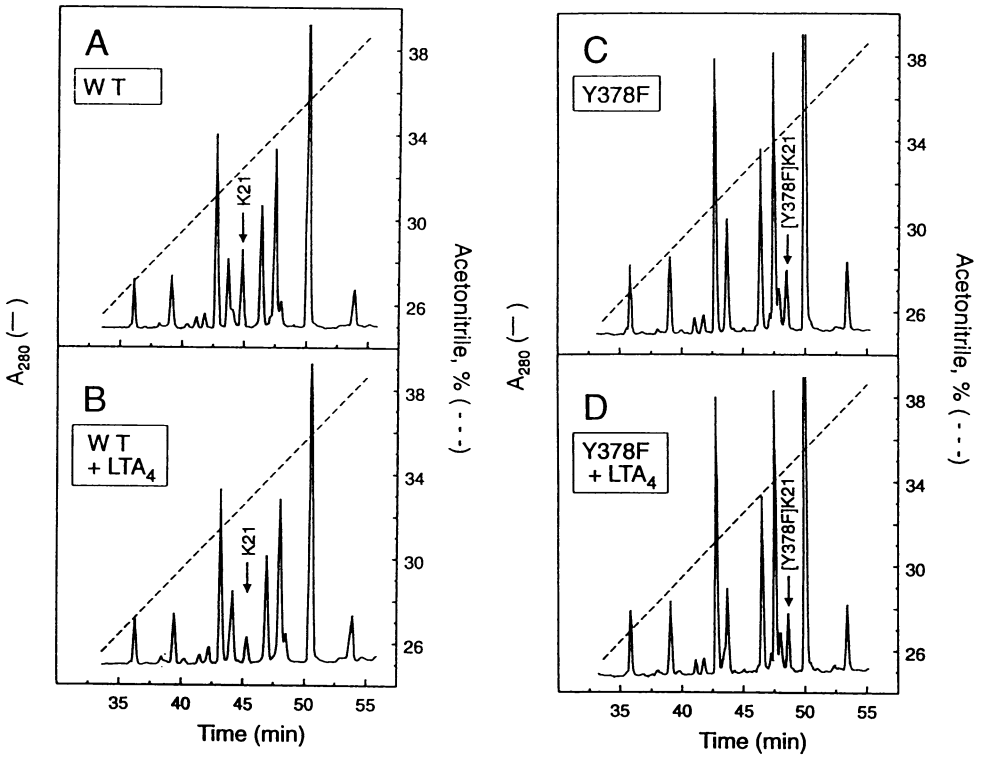

FIG. 6. Differential peptide mapping of untreated and LTA_4-treated wild-type and [Y378F]LTA_4 hydrolase. (*A* and *B*) HPLC chromatograms (30–55 min; 280 nm) of peptides generated by Lys-specific digestion of untreated wild-type LTA_4 hydrolase (*A*) and enzyme suicide inactivated by five additions of 14 μM LTA_4 (*B*). (*C* and *D*) Corresponding HPLC traces obtained with untreated (*C*) [Y378F]LTA_4 hydrolase and [Y378F]LTA_4 hydrolase treated with 5 × 14 μM LTA_4 (*D*). Arrows indicate the position of the unmodified peptides K21 and [Y378F]K21.

DISCUSSION

In a recent study we identified a central peptide segment in LTA_4 hydrolase to which LTA_4 binds covalently during suicide inactivation (15). This segment, denoted peptide K21, was identified by differential peptide mapping and comprises Leu-365 to Lys-385. Furthermore, sequence analysis of a modified form of peptide K21, generated from enzyme treated with LTA_4 ethyl ester, indicated that Tyr-378 is a primary site for the covalent binding of the lipid to the protein. To detail the role of Tyr-378, we replaced this residue with phenylalanine or glutamine. In addition, each of two adjacent and potentially reactive serine residues were exchanged for alanine in separate mutants.

Catalytic Activities of Mutated Enzymes. All four mutated enzymes were catalytically active although the peptidase activities of [Y378Q] and [S379A]LTA_4 hydrolase were substantially reduced suggesting that the phenyl group of Tyr-378 and/or the hydroxyl group of the neighboring Ser-379 may have some function in peptidolysis (Table 2). [Y378F]LTA_4 hydrolase, with the most conservative amino acid replacement at position 378, was selected for further kinetic analysis (Table 3). For the substrate LTA_4, the turnover and the Michaelis constant were increased, yielding an enzyme with only a slightly reduced catalytic efficiency as judged by the k_{cat}/K_m value ($147 \times 10^3\ s^{-1} \cdot M^{-1}$ for the wild-type enzyme versus $90.5 \times 10^3\ s^{-1} \cdot M^{-1}$ for the [Y378F] mutant), which shows that the function of Tyr-378 in the epoxide hydrolase reaction is not catalytic. For the peptidase activity, the mutation led to an increased K_m value for alanine-4-nitroanilide without significantly altering the k_{cat}, and consequently the catalytic efficiency dropped from $447\ s^{-1} \cdot M^{-1}$ of the wild-type enzyme to $58.3\ s^{-1} \cdot M^{-1}$ for the mutant [Y378F].

Mechanism-Based Inactivation of LTA_4 Hydrolase, Protection by Mutation of Tyr-378. Wild-type LTA_4 hydrolase and mutants [S379A] and [S380A] were equally susceptible to inactivation by repeated exposure to LTA_4 (Fig. 5). In contrast, suicide inactivation was essentially abolished in the mutants [Y378F] and [Y378Q]LTA_4 hydrolase, in both of which Tyr-378 has been replaced with residues that are unlikely to react as nucleophils with the allylic leukotriene epoxide. Some modification of residues other than Tyr-378 might still occur because treatment of [Y378Q] with LTA_4 appeared to stimulate the peptidase activity without significantly affecting the epoxide hydrolase activity (Fig. 5). In a different set of experiments with combined activity determinations and peptide mapping, repeated treatment of wild-type and [S380A]LTA_4 hydrolase with LTA_4 ($5 \times 14\ \mu M$) led to approximately 50% inhibition of both enzyme activities, which was also reflected in a corresponding modification of peptides K21 and [S380A]K21. Under the same conditions, [Y378F]LTA_4 hydrolase was neither inactivated nor covalently modified (see Table 4 and Fig. 6).

From kinetic experiments, suicide inactivation of LTA_4 hydrolase has been shown to be compatible with a single type of substrate-enzyme complex that may undergo either turnover or inactivation/covalent modification (12) (see Fig. 1). A constant relationship between these two fates has been established for LTA_4 with a partition ratio (turnover/inactivation) of 129 ± 16 (13). For the mutant [Y378F]LTA_4 hydrolase this relationship is obviously no longer valid. According to the kinetic model in Fig. 1, if still applicable, the partition ratio must be drastically increased, because the turnover has been increased (2.5-fold) and the covalent modification/inactivation prevented. Inasmuch as mechanism-based inactivation is turnover dependent and usually involves modification of a catalytic amino acid residue, the fact that the mutant [Y378F] displayed an intact catalytic efficiency in conjunction with its apparent resistance to inhibition and covalent modification by LTA_4, raises the possibility that suicide inactivation of LTA_4 hydrolase proceeds via a different mechanism.

Possible Function of Tyr-378. From the data of this study we conclude that the two catalytic activities of LTA_4 hydrolase are not critically dependent on Tyr-378. However, both of the mutants, [Y378F] and [Y378Q], produced not only the expected product LTB_4, but also an isomer with a Δ^6-trans, Δ^8-cis configuration of the double bonds (Δ^6-trans, Δ^8-cis-LTB_4), in a yield of 20–30% (M.J.M., M.B., B.S., and J.Z.H, unpublished data). Therefore, one may speculate that Tyr-378 could play a role for the correct positioning of the cis double bond in the conjugated triene system of LTB_4, perhaps by assisting in the proper alignment of LTA_4 in the substrate binding pocket or by promoting a favorable conformation of a putative carbonium ion formed during acid-induced opening of the epoxide ring. Irrespective of a potential role in the enzymatic reaction, it is evident that Tyr-378 is a major structural determinant for suicide inactivation of LTA_4 hydrolase and that removal of its hydroxyl group by a single point mutation generates a recombinant enzyme apparently protected from the catalytic restrictions of this process.

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