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Purification and characterization of leukotriene A_4 hydrolase from human epidermis

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Abstract The leukotriene A_4 hydrolase is a central enzyme in leukotriene B_4 formation. Unlike 5-lipoxygenase, leukotriene A_4 hydrolase activity is present in normal human epidermis, where it is likely to be involved in transcellular leukotriene formation. In this study the leukotriene A_4 hydrolase was purified from human epidermis and human cultured keratinocytes and compared with leukotriene A_4 hydrolase from human neutrophils. To purify leukotriene A_4 hydrolase from human epidermis a new non-specific affinity chromatography column, with the leukotriene A_4 hydrolase inhibitor bestatin coupled to AH-Sepharose, was introduced. The epidermal leukotriene A_4 hydrolase was purified to apparent homogeneity and the molecular weight was determined to be approximately 70,000 Da by SDS-PAGE. The pI was 5.1–5.4 for the epidermal as well as the keratinocyte and neutrophil leukotriene A_4 hydrolase, as determined by chromatofocusing. Only minor differences in the amino acid composition were seen between the three enzyme sources. The optimal pH for the hydrolase activity was 7.5–8.5 for the epidermal and neutrophil leukotriene A_4 hydrolases. Finally, it was also shown that the epidermal leukotriene A_4 hydrolase undergoes suicide inactivation when transforming leukotriene A_4 into leukotriene B_4. It was concluded that there is a close resemblance between the epidermal leukotriene A_4 hydrolase and the hydrolase found in other cell types. Therefore, the human epidermis may be a good model for the in vivo study of transcellular leukotriene formation.

Key words: Leukotriene A_4 hydrolase; Human epidermis; Purification; Eicosanoid

1. Introduction

The biosynthesis of the potent proinflammatory compound leukotriene B_4 (LTB_4) requires the sequential transformation of arachidonic acid by the 5-lipoxygenase (5-LO) and leukotriene A_4 hydrolase, the latter catalyzing the hydrolysis of the unstable epoxide leukotriene A_4 (LTA_4) into LTB_4. Several inflammatory cells, such as polymorphonuclear leukocytes (PMNs), monocytes/macrophages and mast cells, contain the 5-lipoxygenase (5-LO) enzyme as well as the LTA_4 hydrolase, and can, therefore, synthesize LTB_4 from arachidonic acid. Recently, LTA_4 hydrolase activity has been detected in cell types not showing 5-LO activity [1–4]. It has, therefore, been speculated that the LTA_4 hydrolase plays a role in transcellular metabolism, in which LTA_4 is formed in one cell type and then transferred to, and further metabolised in, another cell lacking the 5-LO enzyme [5]. Transcellular metabolism in leukotriene formation has been shown to take place in vitro between monocytes and lymphocytes [6] and between neutrophils and endothelial cells, erythrocytes, platelets and human bronchoalveolar lavage fluid [4,7,8,9]. Recently, we and others have shown that neutrophils and cultured keratinocytes also interact in LTB_4 formation [1,3]. Furthermore, the enzyme has recently been identified and sub-fractionated in human epidermis [10,11].

The LTA_4 hydrolase has been purified and characterized from several different tissues and cell types, such as guinea pig lung [12], guinea pig liver [13], human lung [14,15], human neutrophils [16], human erythrocytes [2], rat neutrophils [17] and the B-lymphocytic cell line Raji [18]. Furthermore, the cDNA clone coding for LTA_4 hydrolase has been isolated from human placenta [19], human spleen [20] and rat mesangial cells [21], and the human LTA_4 hydrolase cDNA has been expressed in *Escherichia coli* [22] and in cultured *Spodoptera frugiperda* insect cells [23].

Recent, results by Bigby et al. [24] have indicated that the kinetic behavior of the LTA_4 hydrolase from transformed human airway epithelial cells (BEAS-2B) is different from that of neutrophils. Furthermore, the human erythrocyte LTA_4 hydrolase [2] varies in terms of molecular weight from the LTA_4 hydrolase from other cell sources. In the guinea pig lung, two catalytically active forms of the LTA_4 hydrolase are found depending on the presence or absence of SH-reducing agents [12]. This dependence was not observed in the human B-lymphocytic cell line Raji [18], although two kinetic patterns of the enzyme were observed. In contrast, the two divergent forms of the LTA_4 hydrolase observed in the Raji cell line were different according to resistance to heat treatment.

The purpose of the present study was to purify and further characterize the epidermal LTA_4 hydrolase. We purified human epidermal and human keratinocyte LTA_4 hydrolase to apparent homogeneity, and compared it to human neutrophil LTA_4 hydrolase with regard to amino acid composition and hydrolase activity. Furthermore, in the purification procedure we introduced a new non-specific affinity chromatography column with the protease inhibitor bestatin coupled to AH-Sepharose. This step takes advantage of the fact that bestatin is a reversible inhibitor of LTA_4 hydrolase.

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Abbreviations: DTT, dithiothreitol; FCS, fetal calf serum; HSA, human serum albumin; KGM, keratinocyte growth medium; 5-LO, 5-lipoxygenase; LT, leukotriene; PAGE, polyacrylamide gel-electrophoresis; PBS, phosphate buffered saline; RP-HPLC, reversed-phase high-performance liquid chromatography; SDS, sodium dodecyl sulfate; Tris-HCl, Trizma hydrochloride.

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2. Materials and methods

2.1. Materials

The LTA_4-methyl ester was obtained from Cascade Biochem Limited (Reading, England). Authentic LTB_4 was from Cayman Chemicals (Ann Arbor, MI). Trizma hydrochloride (Tris-HCl), trypsin, dithiothreitol (DTT) and bestatin were all from Sigma Chemical Co. (St. Louis, MO). Organic solvents were all high-performance liquid chromatography (HPLC) grade from Merck (Darmstad, Germany). Human serum albumin (HSA) was obtained from Nordisk Gentofte (Bagsvaerd, Denmark). The low-calcium keratinocyte growth medium (KGM) from Gibco (Grand Island, NY) was serum free. RPMI 1640 medium was from Gibco, and culture dishes and Centricon-30 were from Life Technologies (European Division, Paisly, Scotland, UK). Fetal calf serum (FCS) was from AH Diagnostics (Aarhus, Denmark). The immobilon-P transfer membrane was from Millipore Corp. (MA, USA). Q-Sepharose (fast flow), AH-Sepharose 4B, phenyl-Sepharose Cl-4B, PBE 94 and polybuffer 74 were all from Pharmacia Fine Chemicals AB (Uppsala, Sweden).

2.2. Preparation of human epidermis

Keratomed skin specimens were obtained from skin samples of patients undergoing plastic surgery as previously described by us [25]. The isolated keratome specimens were chopped into small pieces, homogenized in 50 mM Tris-HCl buffer, 1 mM DTT (pH 7.6) using a Kinematic AG Polytron (3×10 s on ice) and finally centrifuged at $100{,}000 \times g$ at 4°C for 1 h.

2.3. Neutrophil preparation

Human neutrophils were isolated from buffy coats as previously described [26]. After isolation the cells were counted, resuspended in 50 mM Tris-HCl buffer, 1 mM DTT (pH 7.6), ultrasonicated (3×10 s on ice) to produce lysis, and finally centrifuged at $100{,}000 \times g$ at 4°C for 1 h to obtain the cytoplasmic fraction.

2.4. Cultures of human keratinocytes

Cultures of human keratinocytes were prepared as previously described [1]. The cultures were used during the first passage. When sub-confluent, the cell cultures were incubated at 37°C in phosphate buffered saline (PBS) (pH 7.0) containing 0.1% trypsin and 0.1% glucose for 7 min to release the cells. After replacing the incubation medium with RPMI containing 2% FCS, the cells were centrifuged at $800 \times g$ for 10 min and then washed twice in Tris-HCl buffer, 1 mM DTT (pH 7.6) before counting. Finally, the cells were resuspended in Tris-HCl buffer, 1 mM DTT (pH 7.6), ultrasonicated (3×10 s on ice) to produce lysis, and centrifuged at $100{,}000 \times g$ at 4°C for 1 h.

2.5. Purification of LTA_4 hydrolase

All the following procedures were carried out at 4°C.

Step 1. The $100{,}000 \times g$ supernatant of 30 g homogenized epidermis was subjected to ammonium sulphate fractionation. The precipitate formed between 40 and 70% saturation of ammonium sulphate was dissolved in 8 ml of 20 mM Tris-HCl buffer/1 mM DTT (pH 8.3) (buffer A) and then dialyzed against two changes of 1 l of buffer A.

Step 2. The dialyzed sample was applied to a Q-Sepharose column pre-equilibrated with buffer A, and eluted with a 300 ml linear gradient of 0–300 mM KCl in buffer A with a flow rate of 5 ml/min. Fractions were collected at 1 min intervals and assayed for LTA_4 hydrolase activity by determing the capacity to transform LTA_4 (30 nmol) into LTB_4. The active fractions were pooled and the buffer was changed to 10 ml of 50 mM Tris-HCl buffer, 1 mM DTT/125 mM KCl (pH 8.0) (buffer B) using Amicon YM10 to concentrate the sample to 10 ml. To remove the last 10 ml of buffer A a Centricon-30 was used.

Step 3. A column with bestatin coupled to AH-Sepharose 4B was made. This was done in accordance with the principles previously described for affinity chromatography [27]. Briefly, AH-Sepharose equilibrated with 0.5 M NaCl was mixed with a solution containing bestatin dissolved in a mixture of ethanol and water adjusted to pH 4.5. The coupling reaction was initiated by activation of the free amino group on AH-Sepharose by carbodiimide hydrochloride dissolved in water and adjusted to pH 4.5, and the reaction was allowed to take place at room temperature for 24 h. Finally, the excess ligand, urea derivatives and unreacted carbodiimide were washed out with the same mixture of ethanol and water as used for dissolving bestatin.

The collected sample was then applied to the column pre-equilibrated with buffer B and allowed to recirculate over the column for 25 min with a flow rate of 1 ml/min. Then the KCl concentration in buffer B was raised to 175 mM for 12 min before the enzyme was eluted with 500 mM KCl in buffer B. Fractions were collected every 2 min and assayed for LTA_4 hydrolase activity by incubation with LTA_4 (30 nmol) and the active fractions were pooled. Finally the buffer was changed to 0.1 M Tris-HCl, 1 M $(NH_4)_2SO_4$, 1 mM DTT (pH 7.2) (buffer C) using Amicon and Centricon.

Step 4. The enzyme sample was then applied to a phenyl-Sepharose column equilibrated with buffer C, and eluted with a 20 ml linear gradient between buffer C and a 0.1 M Tris-HCl buffer, 1 mM DTT (pH 7.6) with a flow rate of 0.5 ml/min. Fractions were collected at 1 min intervals and assayed for LTA_4 hydrolase activity. The active fractions were collected, the buffer was changed to buffer B, and the sample was re-applied to the column with bestatin coupled to Sepharose in the same way as described in step 3. The enzyme obtained is referred to as the purified human epidermal LTA_4 hydrolase and was stored in 50 mM Tris-HCl buffer, 1 mM DTT (pH 7.6).

When the LTA_4 hydrolase was purified from human neutrophils (10×10^9 cells) and human cultured keratinocytes (10^9 cells) the column with bestatin coupled to Sepharose was not used. With these cells the above mentioned steps 1, 2 and 4 were followed by change of the buffer to 25 mM bis-Tris-HCl buffer, 1 mM DTT (pH 6.3) (buffer D) and application to a PBE 94 column preequilibrated with buffer D. Chromatofocusing was done by eluting (0.5 ml/min) with 10% polybuffer (PBE) 74 adjusted to pH 4 with HCl. The collected active fractions were pooled, the buffer was changed to 50 mM Tris-HCl buffer, 1 mM DTT (pH 7.6), and the enzyme fractions obtained were used in subsequent experiments as the purified LTA_4 hydrolase from human neutrophils and cultured human keratinocytes. Attempts to purify the epidermal LTA_4 hydrolase with these methods were also carried out.

2.6. Assay of LTA_4 hydrolase activity

Free LTA_4 used in the incubations was prepared from LTA_4-methyl ester as previously described [1,28], and the protein content of the different fractions were determined according to Bradford [29] using bovine serum albumin as standard. Variable amounts of protein were preincubated in a total volume of 1 ml of 50 mM Tris-HCl (pH 7.6) containing 1 mM DTT and 1 mg albumin at 37°C for 10 min. Then LTA_4 (30 nmol) in 2 μl MeOH/NaOH was added and incubations carried out for 1 min. Incubations were terminated by the addition of 2 vols. of cold MeOH. Because the free LTA_4 was dissolved in a mixture of MeOH and NaOH, the pH in the incubation medium was raised to 7.8 after the addition of LTA_4. In some experiments the optimal pH for the reaction was investigated, and in these experiments the pH of the buffer was varied so that the final pH was in the range of 5–10. The pH was adjusted with acetate buffer at pH 5.0, with phosphate buffer at pH 6–7, with Tris buffer at pH 7.1–9.0 and with glycine buffer at pH 10.0. In other experiments the enzyme in 50 mM Tris-HCl (pH 7.6) containing 1 mM DTT and 1 mg albumin was incubated at 37°C with LTA_4 (20 nmol) added five times with 15 min between each addition. In separate experiments the pre-incubation time was extended to 1.5 h before incubation with LTA_4 (20 nmol) was carried out. Incubations were terminated by the addition of 2 vols. of cold MeOH.

The typical reaction mixture was kept at −20°C for 20 min and then centrifuged ($1{,}500 \times g$) for 10 min at 4°C to remove precipitated proteins. Lipids from the remaining supernatant were then extracted and prepared for RP-HPLC as previously described [1,26].

2.7. Reverse-phase high-performance liquid chromatography

The extracted lipids were separated by RP-HPLC as previously described [1,26]. The identity of LTB_4 was ascertained as previously described [1] by characteristic ultraviolet absorbance and by chromatographic comparison with authentic LTB_4. Quantitation of LTB_4 was based on integration of optical density and comparison with areas obtained from authentic LTB_4.

2.8. SDS-PAGE gels

SDS-PAGE was carried out on gradient gels (8–15% polyacrylamide gel). Before loading of the sample to the gel, 5 μl of 2% sodium dodecyl sulphate, 10% glycerol, 5% 2-mercaptoethanol, 0.002% bromophenol blue was added per 20 μl sample, and then the samples were boiled for 2 min.

The gel was either silver stained as described by Blum et al. [30], or stained with Coomassie blue R-250. Gels stained with Coomassie blue were blotted to an immobilon-P transfer membrane by semi-dry blotting over 6 h. Then, the band containing the LTA_4 hydrolase was cut out and further analyzed for the amino acid composition.

2.9. Determination of the amino acid composition

Immobilon strips containing the stained hydrolase protein band were subjected to automated amino acid analysis using an applied Biosystems Inc. model 420A amino acid analyzer. In brief, samples were hydrolyzed in 6 N HCl for 75 min at 120°C followed by derivatization to the PITC amino acids. Samples were then quantitatively analyzed by RP-HPLC (note: all steps automated). Quantitation of different samples was made using amino acid standards and a hydrolysis test peptide which contained one of each of the amino acids except Glu, Asp, Cys and Trp. Norleucine was included as an internal standard. The one modification made in the determination of the mol% of each amino acid was that the value for Gly + Cys + Trp was taken as 10% (poor recoveries for Trp- and Cys-PITC are normally encountered). The actual Gly values were usually 2 to 10 times the amount expected and reflects a carry over from the SDS gel and subsequent transfer. 10% is the mean share for the sum of these three amino acids in the average protein. The determination of each amino acid is 5% as judged by multiple runs of the Applied Biosystems test peptide.

3. Results

Purification of the 100,000 × *g* supernatant with ammonium sulphate fractionation, anion-exchange chromatography (Q-Sepharose), hydrophobic chromatography (phenyl-Sepharose) and by chromatofocusing (PBE-94) resulted in a 853-fold purification of neutrophil LTA_4 hydrolase. In contrast, only a 150-fold and a 200-fold purification were obtained for LTA_4 hydrolase from epidermis and cultured keratinocytes, respectively. To get a better purification of the epidermal LTA_4 hydrolase, it was purified by ammonium sulphate fractionation followed by anion-exchange chromatography (Fig. 1), affinity chromatography (Fig. 2), hydrophobic chromatography (Fig. 3) and finally again by affinity chromatography. These purification steps resulted in a 396-fold purification and a yield of 7% (Table 1). These steps resulted in a purification to almost homogeneity of the LTA_4 hydrolase as judged by SDS-PAGE followed by silver staining (Fig. 4). In this method a new affinity chromatography column was introduced. It consisted of bestatin, a LTA_4 hydrolase inhibitor, coupled to Sepharose and was found to be the most efficient step in the purification procedure (Table 1). It can also be seen that the loss over this column was only minor (Table 1), indicating that specific binding of the enzyme

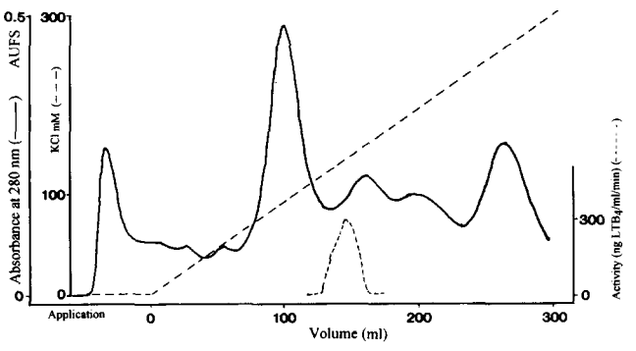

Fig. 1. Anion-exchange chromatography. The enzyme sample was applied to a Q-Sepharose column preequilibrated with buffer A, and then eluted with a 300 ml linear gradient of 0–300 mM KCl in buffer A. 1 min fractions were collected and then assayed for LTB_4 formation capacity (- - -) by incubation with LTA_4(30 nmol).

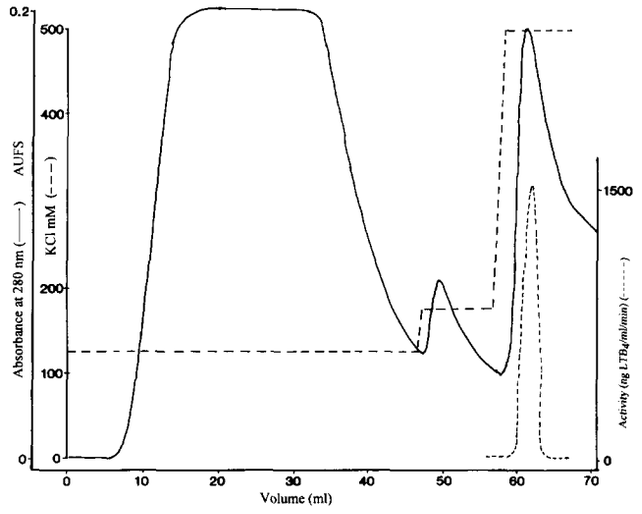

Fig. 2. Affinity chromatography. The enzyme sample was applied to a column with bestatin coupled to AH-Sepharose 4B preequilibrated with buffer B. During the application procedure the sample (10 ml) was allowed to re-circulate over the column for 25 min. Then the KCl concentration in buffer B was raised stepwise to 175 mM and 500 mM and 2 min fractions were collected and assayed for LTB_4 formation capacity (- - -) by incubation with LTA_4 (30 nmol).

to the bestatin took place. This result was supported by the finding of enzyme activity only in the 500 mM KCl eluate fraction (Fig. 2). Furthermore, no enzyme binding took place when the enzyme was applied to AH-Sepharose without bestatin (data not shown). The specific activity in the 100,000 × *g* supernatant of human epidermis was 33.2 ng LTB_4/min/mg (Table 1) corresponding to 0.099 nmol LTB_4/min/mg. The LTA_4 hydrolase activity was 0.18 nmol LTB_4/min/mg in cultured keratinocytes and 0.15 nmol LTB_4/min/mg in neutrophils (data not shown).

The pI of the purified enzyme was 5.5–5.7, as judged by chromatofocusing on the PBE-94 column. No differences were found between the epidermal, the cultured keratinocyte and the neutrophil derived LTA_4 hydrolases. The molecular weight of the enzyme was determined to be approximately 70,000 Da by SDS-PAGE for the epidermal (Fig. 4), the keratinocyte and neutrophil derived LTA_4 hydrolases (data not shown for neutrophils and keratinocytes).

The optimal pH for the transformation of LTA_4 into LTB_4 by the epidermal LTA_4 hydrolase was determined and compared with that of neutrophil derived LTA_4 hydrolase. The optimal pH for the epidermal LTA_4 hydrolase was in the range of 7.5–8.5, and this was the same as for the neutrophil derived LTA_4 hydrolase (data not shown).

Analysis of amino acid composition was done from immobilon-P transfer membranes. This method was used in order to get a higher purity of the sample, because the cultured keratinocyte LTA_4 hydrolase was only purified 200 fold. To allow comparison between the samples from this study, this method was also used in the sample from the epidermis and the neutrophils: the results are shown in Table 2. Small variations between the three samples were found. The most prominent variation was the lower mol% of Pro in the hydrolase obtained from human epidermis and human cultured keratinocytes when compared to human neutrophils.

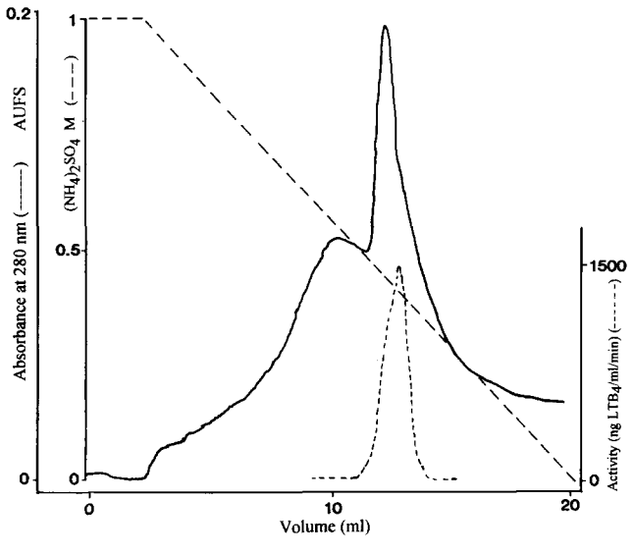

Fig. 3. Hydrophobic chromatography. The enzyme sample was applied to a phenyl-Sepharose column preequilibrated with buffer C. The enzyme was then eluted with a 20 ml linear gradient between buffer C and a 0.1 M Tris-HCl buffer, 1 mM DTT (pH 7.6). 1 min fractions were collected and assayed for LTA_4 hydrolase activity (- - -) by incubation with LTA_4 (30 nmol).

In order to elucidate whether the epidermal LTA_4 hydrolase undergoes suicide inactivation when transforming LTA_4 into LTB_4, LTA_4 was added to the purified epidermal LTA_4 hydrolase repeatedly 5 times at 15 min intervals. It was found that the velocity of LTB_4 formation decreased each time LTA_4 was added, resulting in almost no new LTB_4 formation after the last LTA_4 addition (Fig. 5). The same phenomenon was observed with the neutrophil LTA_4 hydrolase (data not shown). The purified LTA_4 hydrolase was also preincubated for 1.5 h before LTA_4 was added and the initial velocity was determined. The initial velocity was not different from that found in Fig. 5 (data not shown). These observations suggest that the epidermal and the neutrophil LTA_4 hydrolases undergo suicide inactivation when transforming LTA_4 into LTB_4.

4. Discussion

Although LTA_4 hydrolase activity has previously been demonstrated in human epidermis [10,11] and in human cultured keratinocytes [1,3], this is the first time that the human epidermal and human cultured keratinocyte LTA_4 hydrolase has been purified and characterized. In contrast to neutrophil LTA_4 hydrolase, the purification of epidermal LTA_4 hydrolase required the introduction of a new non-specific affinity chromatography column. The affinity column which contained bestatin coupled to AH-Sepharose was used for the first time to purify LTA_4 hydrolase and found to be very efficient. The reason for using bestatin in the purification procedure is that bestatin has previously been shown to be a potent reversible inhibitor of LTA_4 hydrolase [31,32]. It is interesting that LTA_4 hydrolase can be bound to bestatin and then released by raising the KCl concentration. The LTA_4 hydrolase enzyme has recently been described as a bi-functional enzyme exhibiting both hydrolase activity and peptidase activity [33–35]. Furthermore, sequence comparison with certain zinc metalloenzymes, including aminopeptidase N, revealed the presence of a zinc-binding motif in the LTA_4 hydrolase [36]. Bestatin is known as a potent inhibitor of many aminopeptidases, including the LTA_4 hydrolase [31,32], and it has been suggested that bestatin mimics the tetrahedral intermediate of hydrolysis using the C-2 OH to form a complex with the active-site Zn^{2+} of these aminopeptidases [37]. In developing the affinity column, it was speculated that the bestatin could be coupled through its carboxy group to the AH-Sepharose resulting in an amide bond. This would keep the hydroxy group free to interact with the Zn^{2+}. Steric hindrance should be avoided since the AH-Sepharose contains a 6 carbon spacer arm which is very flexible. The bestatin column might also be useful in the separation of active LTA_4 hydrolase from inactivated LTA_4 hydrolase. With recombinant enzyme and enzyme purified from leukocytes it has been shown that the inactivation caused by LTA_4 is a result of a covalent modification resulting in a LTA_4–enzyme complex [38]. Furthermore, bestatin can inhibit this covalent modification, indicating that LTA_4 and bestatin act at the same active site of the enzyme. Therefore, inactivated LTA_4 hydrolase might not be able to bind to the bestatin column in the same way as active LTA_4 hydrolase.

Human neutrophils, which have previously been used as a cell source for the purification of LTA_4 hydrolase [16], were included in this study to allow a direct comparison with the epidermal and keratinocyte LTA_4 hydrolase with a previously characterized enzyme. In Table 2 the findings in this study are compared with studies of other human cell types. In terms of molecular weight, the keratinocyte and epidermal LTA_4 hydrolases are similar to human neutrophil, human lung and human recombinant hydrolases, but different from the human erythrocyte hydrolase. The findings of a molecular weight of approximately 70,000 Da is also in accordance with the findings in guinea pig lung [12], rat neutrophils [17] and in the human B-lymphocytic cell line Raji [18]. The lower molecular weight of the erythrocyte LTA_4 hydrolase might reflect the presence of different isoforms of the enzyme, as speculated by Samuels-

Table 1
Purification of LTA_4 hydrolase from human epidermis

Step	Total protein (mg)	Total activity (ng LTB_4/min)	Specific activity (ng LTB_4/min/mg)	Yield (%)	Purification (fold)
100,000 × g supernatant	510.1	16953.1	33.2	100	1
Ammonium sulphate	228.0	9152.0	40.1	54	1.2
Q-Sepharose	13.4	4530.0	338.1	27	10.2
Bestatin coupled to Sepharose	1.114	3989.3	3581.1	24	108
Phenyl-Sepharose	0.206	2115.3	10268.4	12	309
Bestatin coupled to Sepharose	0.096	1263.6	13162.5	7	396

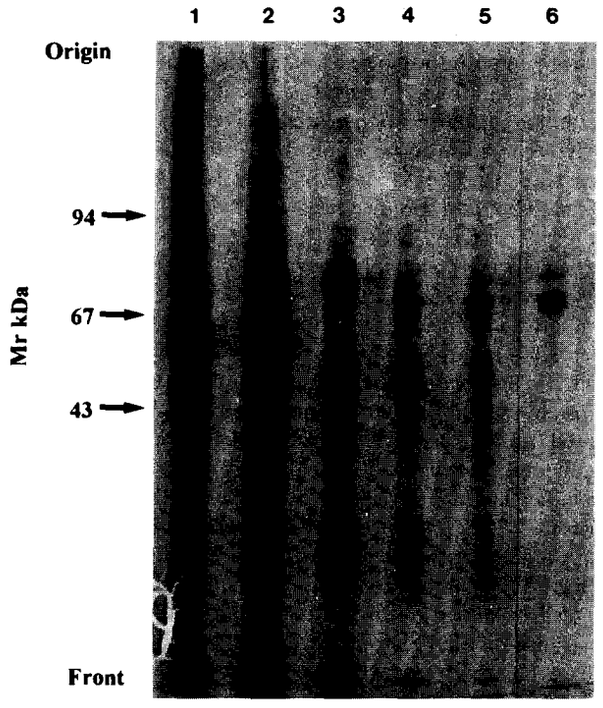

Fig. 4. SDS-PAGE of the different steps in the purification of LTA_4 hydrolase from human epidermis. The gel was silver stained. Lane 1, 100,000 × *g* supernatant. Lane 2, 40–70% ammonium sulphate precipitation. Lane 3, Q-Sepharose. Lane 4, bestatin coupled to AH-Sepharose 4B (first run). Lane 5, phenyl-Sepharose. Lane 6, bestatin coupled to AH-Sepharose 4B (second run).

son et al. [39]. It could also represent a breakdown product of the intact hydrolase, as shown by Orning et al. [40]. Recently, Rådmark et al. have shown that the erythrocyte LTA_4 hydrolase has a MW close to 70,000 Da [41].

The pI of 5.1–5.4 found in all three enzyme preparations was obtained with the SH-reducing agent DTT present in the buffer. The pI was not determined with the absence of SH-reducing agents in buffers, although Bito et al. [12] have shown that two catalytically active forms of the LTA_4 hydrolase, dependent on the presence or absence of SH-reducing agents, are present in guinea pig lung. Haeggström et al. have also shown that guinea pig liver LTA_4 hydrolase is eluted as two peaks in chromatofocusing differing by 0.4 in their pI if not pretreated with DTT [13]. The pI observed in this study is in good accordance with the findings for the LTA_4 hydrolase in human neutrophils and human lung (Table 2). Furthermore, no differences were seen in the pH optimum for the hydrolase activity of the enzyme derived from epidermis and neutrophils. The optimal pH was slightly alkaline, as has been shown with the enzyme from other sources (Table 2).

In this study we also showed that the epidermal LTA_4 hydrolase, similar to neutrophil and erythrocyte LTA_4 hydrolase [2,38], undergoes suicide inactivation when transforming LTA_4 into LTB_4. This is in contrast to what has recently been reported for transformed human airway epithelial cells (BEAS-2B) by Bigby [24]. The human airway epithelial cell derived LTA_4 hydrolase is also different from human leukocyte [16], human epidermal [10] and human erythrocyte [2] derived LTA_4 hydrolases in that it does not obey Michaelis–Menten saturation kinetics. The inactivation of the enzyme is not a result of degradation of the enzyme during the incubation period, while pre-incubation of the LTA_4 hydrolase for 1.5 h did not lower the initial velocity of the transformation of LTA_4 into LTB_4.

Only small variations in the amino acid composition were seen in the samples analyzed in this study (Table 2), and the determined amino acid composition bore a close resemblance to that determined in other studies (Table 2), although the Ser determined in this study was higher than determined previously. However, the Ser level in the sample from the epidermis and the cultured keratinocytes was not different from that of human neutrophils, which was our control. Even in the study by Odlander et al. [18], in which two kinetic patterns of the enzyme were found, no difference in the amino acid composition between the two forms was seen, and compared with our observations only minor differences were seen. It is interesting to note that the amino acid composition calculated from the cDNA of the LTA_4 hydrolase from human spleen varies regarding Asp and Glu from what has been determined in other studies (Table 2).

The specific activity observed in this study is in good accordance with the 0.072 nmol LTB_4/min/mg found in erythrocytes [2], but much lower than the 0.85 nmol LTB_4/min/mg found in guinea pig lung [12].

In this study we have shown that the epidermal LTA_4 hydrolase bears a close resemblance in amino acid composition and hydrolase kinetics to the LTA_4 hydrolase obtained from almost all other cell types, except the airway epithelial derived LTA_4 hydrolase [24]. Because of this close resemblance to other cell types, and because the epidermal LTA_4 hydrolase undergoes suicide inactivation when transforming LTA_4 into LTB_4, inflamed human epidermis may be a good in vivo model for studying the transcellular metabolism in leukotriene formation.

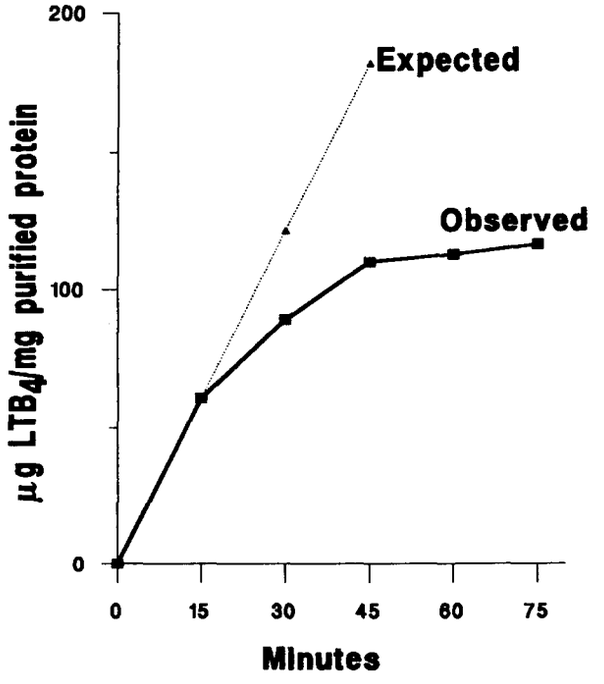

Fig. 5. Purified LTA_4 hydrolase was incubated with LTA_4 (20 nmol) added repeatedly at 15 min intervals. The results represent the mean of two experiments.

Table 2
Properties of LTA_4 hydrolase from various human sources

Source	Human epidermis	Human cultured keratinocytes	Human neutrophils	Human leukocytes	Human lung	Human erythrocytes	Human cDNA[2]
Molecular weight[1]	69,000–70,000	69,000–70,000	69,000–70,000	68,000–70,000	68,000–71,000	54,000–1,000[3]	69,154
pI	5.1–5.4	5.1–5.4	5.1–5.4	5.1–5.7	5.1–5.3	4.9 ± 0.2	6.1
pH optimum	7.5–8.5	ND	7.5–8.5	8.0–9.0	7.8–9.0	7.0–8.0	ND
Amino acid composition (mol %)							
As	7.0	8.9	10.4	9.8	9.9		9.0
Gl	11.0	12.2	14.0	10.1	13.0		10.9
Ser	13.3	15.9	16.3	8.2	5.3		7.4
Gly	–	–	–	5.5	5.3		4.9
His	2.0	1.8	1.8	3.0	2.9		2.6
Arg	5.7	4.9	5.9	3.2	4.1		3.8
Thr	4.9	4.3	4.2	7.0	6.6	ND	6.6
Ala	9.7	6.1	6.0	6.7	6.8		6.2
Pro	2.5	3.6	6.9	6.3	6.5		5.7
Tyr	3.6	2.9	3.4	3.4	3.7		3.6
Val	6.7	4.4	3.7	7.0	5.7		6.2
Met	2.7	1.9	–	1.6	1.9		1.8
Ile	5.6	4.4	3.2	3.9	5.0		5.1
Leu	11.8	10.1	6.7	11.8	12.0		11.3
Phe	3.5	4.1	2.1	4.8	4.7		4.4
Lys	–	4.6	5.2	7.6	6.8		6.6
Cys	–	–	–	–	–		1.8
Tyr	–	–	–	–	–		2.1
Reference	This study	This study	This study	16	14	2	20

[1]Determined by SDS-PAGE.
[2]Calculated from human cDNA.
[3]May represent a break down product (see text).
ND = Not determined.

References

[1] Iversen, L., Fogh, K., Ziboh, V.A., Kristensen, P., Schmedes, A. and Kragballe, K. (1993) J. Invest. Dermatol. 100, 293–298.
[2] McGee, J. and Fitzpatrick, F. (1985) J. Biol. Chem. 260, 12832–12837.
[3] Solá, J., Godessart, N., Vila, L., Puig, L. and de Moragas, J.M. (1992) J. Invest. Dermatol. 98, 333–339.
[4] Claesson, H.E. and Haeggström, J.Z. (1988) Eur. J. Biochem. 173, 93–100.
[5] Claesson, H.E., Haeggström, J., Odlander, B., Medina, J.F., Wetterholm, A., Jakobsson, P.J. and Rådmark, O. (1991) in: Cell–cell Interactions in the Release of Inflammatory Mediators (Wong, P.Y.K. and Serhan, C.N. eds.) pp. 307–315, Plenum Press, New York.
[6] Jakobsson, P.J., Odlander, B. and Claesson, H.E. (1991) Eur. J. Biochem. 196, 395–400.
[7] McGee, J.E. and Fitzpatrick, F.A. (1986) Proc. Natl. Acad. Sci. USA 83, 1349–1353.
[8] Maclouf, J.A. and Murphy, R.C. (1988) J. Biol. Chem. 263, 174–181.
[9] Munafo, D.A., Shindo, K., Baker, J.R. and Bigby, T.D. (1994) J. Clin. Invest. 93, 1042–1050.
[10] Iversen, L., Ziboh, V.A., Shimizu, T., Ohishi, N., Rådmark, O., Wetterholm, A. and Kragballe, K. (1994) J. Dermatol. Sci. 7, 191–201.
[11] Ikai, K., Okano, H., Horiguchi, Y. and Sakamoto, Y. (1994) J. Invest. Dermatol. 102, 253–257.
[12] Bito, H., Ohishi, N., Miki, I., Minami, M., Tanabe, T., Shimizu, T. and Seyama, Y. (1989) J. Biochem. 105, 261–264.
[13] Haeggström, J., Bergman, T., Jörnvall, H. and Rådmark, O. (1988) Eur. J. Biochem. 174, 717–724.
[14] Ohishi, N., Izumi, T., Minami, M., Kitamura, S., Seyama, Y., Ohkawa, S., Terao, S., Yotsumoto, H., Takaku, F. and Shimizu, T. (1987) J. Biol. Chem. 262, 10200–10205.
[15] Ohishi, N., Izumi, T., Seyama, Y. and Shimizu, T. (1990) Methods Enzymol. 187, 286–295.
[16] Rådmark, O., Shimizu, T., Jörnvall, H. and Samuelsson, B. (1984) J. Biol. Chem. 259, 12339–12345.
[17] Evans, J.F., Dupuis, P. and Ford-Hutchinson, A.W. (1985) Biochim. Biophys. Acta 840, 43–50.
[18] Odlander, B., Claesson, H.E., Bergman, T., Rådmark, O., Jörnvall, H. and Haeggström, J.Z. (1991) Arch. Biochem. Biophys. 287, 167–174.
[19] Funk, C.D., Rådmark, O., Fu, J.Y., Matsumoto, T., Jörnvall, H., Shimizu, T. and Samuelsson, B. (1987) Proc. Natl. Acad. Sci. USA 84, 6677–6681.
[20] Minami, M., Ohno, S., Kawasaki, H., Rådmark, O., Samuelsson, B., Jörnvall, H., Shimizu, T., Seyama, Y. and Suzuki, K. (1987) J. Biol. Chem. 262, 13873–13876.
[21] Makita, N., Funk, C.D., Imai, E., Hoover, R.L. and Badr, K.F. (1992) FEBS Lett. 299, 273–277.
[22] Minami, M., Minami, Y., Emori, Y., Kawasaki, H., Ohno, S., Suzuki, K., Ohishi, N., Shimizu, T. and Seyama, Y. (1988) FEBS Lett. 229, 279–282.
[23] Gierse, J.K., Luckow, V.A., Askonas, L.J., Duffin, K.L., Aykent, S., Bild, G.S., Rodi, C.P., Sullivan, P.M., Bourner, M.J., Kimack, N.M. and Krivi, G.G. (1993) Protein Exp. Purif. 4, 358–366.
[24] Bigby, T.D. (1992) Chest 101, 33.
[25] Iversen, L., Kristensen, P., Grøn, B., Ziboh, V.A. and Kragballe, K. (1994) Arch. Dermatol. Res. 286, 261–267.
[26] Fogh, K., Herlin, T. and Kragballe, K. (1988) Arch. Dermatol. Res. 280, 430–436.
[27] Pharmacia (1988) Affinity Chromatography Principles and Methods, Pharmacia LKB Biotechnology, Uppsala, Sweden.
[28] Maycock, A.L., Anderson, M.S., De Sousa, D.M. and Kuehl Jr., F.A. (1982) J. Biol. Chem. 257, 13911–13914.
[29] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[30] Blum, H., Beier, H. and Gross, H.J. (1987) Electrophoresis 8, 93–99.

[31] Orning, L., Krivi, G. and Fitzpatrick, F.A. (1991) J. Biol. Chem. 266, 1375–1378.
[32] Evans, J.F. and Kargman, S. (1992) FEBS Lett. 297, 139–142.
[33] Minami, M., Ohishi, N., Mutoh, H., Izumi, T., Bito, H., Wada, H., Seyama, Y., Toh, H. and Shimizu, T. (1990) Biochem. Biophys. Res. Commun. 173, 620–626.
[34] Wetterholm, A., Medina, J.F., Rådmark, O., Shapiro, R., Haeggström, J.Z., Vallee, B.L. and Samuelsson, B. (1992) Proc. Natl. Acad. Sci. USA 89, 9141–9145.
[35] Haeggström, J.Z., Wetterholm, A., Vallee, B.L. and Samuelsson, B. (1990) Biochem. Biophys. Res. Commun. 173, 431–437.
[36] Toh, H., Minami, M. and Shimizu, T. (1990) Biochem. Biophys. Res. Commun. 171, 216–221.
[37] Nishizawa, R., Saino, T., Takita, T., Suda, H., Aoyagi, T. and Umezawa, H. (1977) J. Med. Chem. 20, 510–515.
[38] Orning, L., Gierse, J., Duffin, K., Bild, G., Krivi, G. and Fitzpatrick, F.A. (1992) J. Biol. Chem. 267, 22733–22739.
[39] Samuelsson, B. and Funk, C.D. (1989) J. Biol. Chem. 264, 19469–19472.
[40] Orning, L., Jones, D.A. and Fitzpatrick, F.A. (1990) J. Biol. Chem. 265, 14911–14916.
[41] Rådmark, O. and Haeggström, J. (1990) in: Advances in Prostaglandin, Thromboxane and Leukotriene Research, vol. 20 (Samuelsson, B., Dahlen, S.E., Fritch, J., Hedquist, P. eds.) pp. 35–45, Raven Press, New York.