Biochem. J. (1992) 288, 831–837 (Printed in Great Britain)

Butyric acid-induced differentiation of HL-60 cells increases the expression of a single lysophospholipase

Diane GARSETTI,* Frederick HOLTSBERG,* Marion R. STEINER,† Robert W. EGAN* and Mike A. CLARK*‡

*Schering–Plough Research, 60 Orange Street, Bloomfield, NJ 07003, U.S.A., and †University of Kentucky, Lexington, KY 40536, U.S.A.

Treatment of HL-60 cells with 0.5 mM-butyric acid resulted in morphological changes, including the formation of cytoplasmic granules, nuclear condensation and segmentation. These differentiated cells had an elevated phospholipase A_2 activity and an increased capacity to synthesize a variety of eicosanoids, including both lipoxygenase and cyclo-oxygenase products. Phospholipase A_2-mediated release of arachidonic acid is accompanied by an equimolar production of potentially cytotoxic lysophospholipid. In association with the differentiation process, there was a 2–3-fold increase in lysophospholipase activity. Subsequent studies were undertaken to identify and characterize the lysophospholipases in this cell system, with 1-[1-^{14}C]palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine as substrate. Hydrophobic chromatography of both undifferentiated and differentiated cell extracts revealed three peaks of enzyme activity. Extracts of differentiated cells contained a dramatic increase in activity contained in peak 2. The increase in enzymic activity of peak 2 appeared to account for the increase in total lysophospholipase activity found in the differentiated cell homogenates. The lysophospholipases contained in peaks 2 and 3 were purified to homogeneity and were 20 and 22 kDa respectively, as determined by denaturing polyacrylamide-gel electrophoresis. Peaks 2 and 3 were similar on the basis of amino acid composition, but had distinctive *C*-terminal peptide amino acid sequences. Enzymic characterization of these proteins demonstrated that there was no detectable level of non-specific esterase, acyltransferase or transacylase activity associated with these proteins. We concluded that peak 2 lysophospholipase is regulated by differentiation in HL-60 cells and may play an important role in protecting these cells from the cytolytic effects of the lysophospholipids produced by the activation of phospholipase A_2.

INTRODUCTION

During inflammation, leukocytic cells proliferate, become terminally differentiated, activated, and infiltrate the affected tissue. Granulocytes and macrophages, acting in concert, produce a variety of proinflammatory mediators such as cytokines and eicosanoids, which serve to perpetuate and exacerbate the inflammatory response. The HL-60 cell line, derived from a human leukaemia, is a commonly used myeloid progenitor cell line capable of differentiating into granulocytes or macrophages [1,2]. HL-60 cells are frequently chosen as a model system for studying the terminal differentiation processes and cellular metabolism of leukocytic cells. Butyric acid has been used to induce the differentiation of HL-60 cells [3]. Butyric acid-treated HL-60 cells express many of the phenotypic features of the differentiated granulocyte, including a condensed and segmented nucleus, and a cytoplasm filled with numerous granules [3].

Differentiated HL-60 cells have increased capacity for the production of a number of proinflammatory mediators, including eicosanoids [4–6]. Phospholipase A_2 activity has also been shown to increase upon differentiation of the HL-60 cells [7]. The rate-limiting step in eicosanoid production is the release of non-esterified arachidonic acid from the phospholipid via phospholipase A_2 [8]. Phospholipase A_2 hydrolysis of phospholipids produces equal molar amounts of lysophospholipid and non-esterified fatty acid. The lysophospholipids themselves have been implicated in signal transduction [9] and are potentially cytotoxic at high concentrations [10]. Therefore it is important that the cells carefully regulate the levels of the lysophospholipids produced by phospholipase A_2.

Lysophospholipids are degraded by the lysophospholipases. Several different lysophospholipases have been isolated from various tissues, including heart, pancreas, liver and amnion [11–13], and from cells including eosinophils [14] and macrophages [15–17]. In fact, two different murine macrophage cell lines have been shown to possess multiple lysophospholipases [15–17]. One of these enzymes is of molecular mass 110 kDa and has multiple enzymic activities, including phospholipase A_2 activity [17,18]. These macrophage cells also possess two small-molecular-mass lysophospholipases (28 and 27 kDa), which have similar enzyme characteristics, yet appear to be immunologically distinct proteins [15,16]. The roles of each of these isoenzymes are not yet known, and to obtain a better understanding of them we have chosen the differentiation of the HL-60 cell line as a model system for studying the regulation of lysophospholipases.

MATERIALS AND METHODS

Materials

The substrate used in these assays was 1-[1-^{14}C]palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine (New England Nuclear, Boston, MA, U.S.A.). Non-radioactive synthetic 1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine from Avanti (Pelham, AL, U.S.A.) was used to lower the specific radioactivity of the substrate to 0.2 mCi/mmol. The substrate for phospholipase A_1/A_2 assay was 1,2-dipalmitoyl-*sn*-3-glycerophospho[*N*-*methyl*-^{3}H]choline (Amersham, Arlington Heights, IL, U.S.A.). *p*-Nitrophenyl acetate and palmitoyl- and oleoyl-CoA were obtained from Sigma (St. Louis, MO, U.S.A.). The radioimmunoassay kits for quantifying prostaglandin E_2 and 5- and 15-hydroxy-

Abbreviation used: ME, 2-mercaptoethanol.

‡ To whom all correspondence should be addressed.

eicosatetraenoic acid were obtained from Advanced Magnetics (Cambridge, MA, U.S.A.), and those for leukotrienes B_4 and C_4 were obtained for New England Nuclear (Boston, MA, U.S.A.).

Cells and tissue culture

The HL-60 cell line used in these experiments was generously provided by Dr. David W. Golde (Department of Medicine, University of California at Los Angeles). These cells were grown in Iscove's medium (Gibco, Grand Island, NY, U.S.A.) supplemented with fetal-bovine serum (10%, v/v) (Hyclone, Logan, UT, U.S.A.). The undifferentiated cells used in these experiments were exponentially growing cultures of HL-60 cells. Terminal differentiation was achieved by incubating the cells with 0.5 mM-butyric acid for 7 days as previously described [19].

Purification of the lysophospholipase

Cells were harvested by centrifugation at 10000 *g* in a zonal rotor (Sorvall, Wilmington, DE, U.S.A.). The resulting cell pellet was washed with phosphate-buffered saline and the cells were frozen at −70 °C until used. There was no appreciable loss of enzymic activity for periods of up to 1 month under these storage conditions. The frozen cell pellets were thawed in 10 vol. of 0.1 M-Tris (pH 8.0)/1 M-$(NH_4)_2SO_4$/10 mM-2-mercaptoethanol (ME)/2 mM-EDTA and then disrupted by sonication at 4 °C with a Branson (Danbury, CT, U.S.A.) sonicator set at maximum intensity for 30 s. The cell homogenate was clarified by centrifugation at 10000 *g* for 20 min at 4 °C. The resulting supernatant was then applied to the hydrophobic column.

Hydrophobic chromatography of lysophospholipases

The high-speed supernatant was applied to a 50 mm × 10 cm Ether-5 PW hydrophobic column (TosoHaas, Philadelphia, PA, U.S.A.) at a flow rate of 20 ml/min. The column was eluted by decreasing the $(NH_4)_2SO_4$ concentration from 1 M to 0 linearly over a 30 min time period, and a flow rate of 20 ml/min was used throughout the chromatography. Fractions were collected at 1 min intervals and assayed for lysophospholipase activity.

Anion-exchange chromatography of lysophospholipases

Peaks 2 and 3 from the hydrophobic column were extensively dialysed against a buffer containing 0.1 M-Tris, pH 8.0, 10 mM-ME and 2 mM-EDTA, and then further purified by anion-exchange chromatography. An MA7P anion-exchange column (21 cm × 10 cm) from Bio-Rad (Richmond, CA, U.S.A.) was pre-equilibrated with the same dialysis buffer, and the samples were applied at a flow rate of 5 ml/min. The column was eluted with a linear gradient of 0–2 M-NaCl in the same buffer. The duration of the gradient was 30 min, and a flow rate of 5 ml/min was used throughout this chromatographic step. Fractions were collected at 1 min intervals and assayed for lysophospholipase activity.

Gel-filtration chromatography of lysophospholipases

The resulting lysophospholipase activities obtained from the anion-exchange columns were concentrated to approx. 0.4 ml by ultrafiltration on YM10 membranes (Amicon, Beverly, NY, U.S.A.). The concentrated samples were next applied to two gel-filtration columns linked in tandem (GF 250 and GF 450; Zorbax, MacMod, Wilmington, DE, U.S.A.). The mobile phase consisted of 0.5 M-NaCl, 0.1 M-Tris, pH 8.0, 10 mM-ME and 2 mM-EDTA. A flow rate of 1 ml/min was used throughout the chromatography. Fractions were collected at 1 minute intervals and assayed for enzymic activity.

Protein determination

Protein determinations were done by the method of Bradford [20], with dye reagent obtained from Bio-Rad. BSA (Sigma) was used as a standard.

Lysophospholipase activity

Lysophospholipase activity was measured in a buffer consisting of 0.1 M-Tris, pH 8.0, 10 mM-ME and 2 mM-EDTA, with a reaction volume of 0.5 ml and an incubation temperature of 37 °C. The reactions were initiated by addition of the radiolabelled substrate (250 μM final concn.), incubated for 1 h, then terminated by extraction of the non-esterified fatty acid with Dole reagent as previously described [16]. All three lysophospholipase enzymes isolated from HL-60 cells had pH optima of 8.0 as measured by this assay. Under the assay conditions used, all reactions were linear with respect to time and protein concentration.

Non-specific esterase activity

The purified enzymes were assayed for non-specific esterase with *p*-nitrophenyl acetate (66 mM) (Sigma). The buffer used was 0.1 M-phosphate, pH 7.0, and the incubations were carried out at 30 °C for 30 min. The absorbance was determined at 660 nm. Cholesterol esterase (Sigma) was used as a positive control.

Transacylase activity

The purified lysophospholipases were also assayed for transacylase activity as assessed by the formation of radioactive phosphatidylcholine as previously described [16]. The reaction product, phosphatidylcholine, was separated from the lysophosphatidylcholine substrate by t.l.c. (silica gel G plates; Analtech, Wilmington, DE, U.S.A.) with a mobile phase consisting of chloroform/methanol/acetic acid/water (25:15:4:2, by vol.). The regions of the plate corresponding to phosphatidylcholine and lysophosphatidylcholine were made visible by using iodine vapour, then scraped off, and the radiolabel was quantified by scintillation spectroscopy.

Acyltransferase activity

The purified lysophospholipases were assayed for acyltransferase activity under the same assay conditions as were used for the lysophospholipase determinations, with addition of 2 mM-$MgCl_2$ and 125 μM-palmitoyl-CoA or -oleoyl-CoA. Phosphatidylcholine generation was quantitated by t.l.c. using the conditions described above for the transacylase assay. Crude cell homogenates were used as a positive control.

Phospholipase A_1/A_2 activity

The phospholipase A_1/A_2 activity in the purified enzyme preparations, as well as in the total sonicated-cell preparation, was quantified by methods previously described [21]. Briefly, this assay consists of [^{3}H]phosphatidylcholine radiolabelled in the polar head group and separation of the lysophosphatidylcholine product from the substrate by t.l.c. The chromatographic conditions used are those described above for the transacylase assay. The assay buffer was 0.1 M-Tris, pH 9.0, 100 mM-NaCl and 1 mM-$CaCl_2$. Reaction time was 60 min and the reactions were carried out at 37 °C. Phospholipase reactions were quenched by Folch extraction of the phospholipids [22].

Radioimmunoassays

Quantification of the various eicosanoids produced by both undifferentiated and differentiated cells were determined identically. Cells were first rinsed in PBS and resuspended at a final concentration of 10^6 cells/ml in PBS. Cells were incubated at

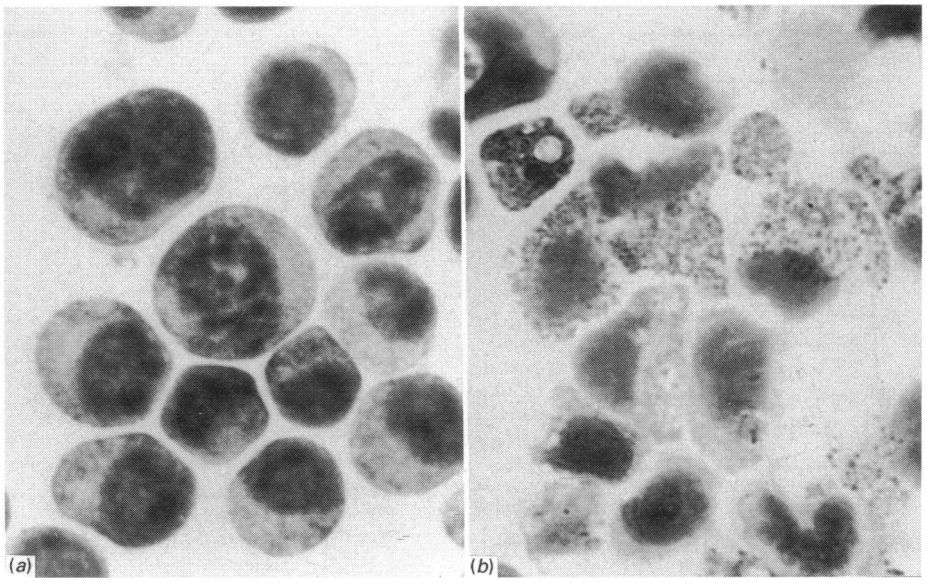

Fig. 1. Differentiation of HL-60 cells

Cells were grown to a density of 1×10^6 cells/ml, then induced to differentiate by treatment with butyric acid (0.5 mM) for 7 days. Undifferentiated (*a*) and differentiated (*b*) cells were stained with Wright's stain and are shown above. Final magnification approx. $\times 2000$.

37 °C for 10 min in either PBS alone or PBS containing the Ca^{2+} ionophore A23187 (Sigma) (1 μM). Next the cells were removed from the saline by centrifugation (2000 *g* for 10 min) and the cell-free supernantants were assayed for the identical eicosanoids as suggested by the manufacturer of the radioimmunoassay kits.

Amino acid analysis, peptide digests and protein sequencing

Amino acid compositions of purified peaks 2 and 3 were determined by hydrolysing the proteins with HCl, and preparing the phenylthiocarbamoyl derivatives of the amino acids. These derivatives were then separated by reverse-phase h.p.l.c. and quantified by using Waters (Boston, MA, U.S.A.) Picotag reagents and h.p.l.c. column as suggested by the manufacturer.

The *C*-terminal peptides were isolated by using immobilized anhydrotrypsin columns obtained from Pierce (Rockford, IL, U.S.A.), and used as directed by the manufacturer. The resulting peptides were sequenced by covalent attachment [23] to Sequelon-Aryl-Amine membranes (Milligen, Boston, MA, U.S.A.) as suggested by the manufacturer. Automated Edman degradation and amino acid phenylthiohydantoin analyses were carried out on a model 6625 sequencer (Milligen).

RESULTS

Treatment of HL-60 cells with 0.5 mM-butryic acid resulted in morphological changes in the cells, including formation of cytoplasmic granules, nuclear condensation and segmentation (Fig. 1). Associated with these events was an increase in the synthesis of a variety of eicosanoids, as shown in Table 1. The differentiated cells, when treated with Ca^{2+} ionophore, also had increased capacity to release these eicosanoids. The differentiated cells also had a 3–4-fold increase in phospholipase A_2 activity as measured in cell-free assays with phosphatidylcholine as the substrate (Table 2). Sonicated preparations from differentiated cells also had a concomitant 2–3-fold increase in the total lysophospholipase activity (Table 2). Thus, as the HL-60 cells differentiate, there is an increased eicosanoid production, an increased phospholipase A_2 activity and an increase in total lysophospholipase activity.

The lysophospholipase activity of the HL-60 cell homogenates was resolved into three distinct peaks of activity when subjected to hydrophobic interactive chromatography (Fig. 2). These activities are hereafter referred to as peaks 1, 2 and 3, based on their order of elution from this column. Comparison of the differentiated-cell and non-differentiated-cell lysophospholipase activities eluted from the hydrophobic column demonstrated that the differentiated-cell extracts contained substantially more peak 2 lysophospholipase activity (Fig. 2). The undifferentiated-cell extracts contained approx. 7% of the total enzyme activity in the peak 2 fraction. In contrast, approx. 60% of the lysophospholipase activity in extracts from differentiated cells

Table 1. Eicosanoid production in HL-60 cells

All values are expressed in pg/ml per 10^6 cells. The data shown represent the means of four separate experiments. All experiments were assayed in duplicate and the standard errors were < 15%. ND, not detected (< 0.2 pg/ml). Abbreviations: PG, prostaglandin; LT, leukotriene; HETE, hydroxyeicosatetraenoic acid.

Cells	A23187 (1 μM)	PGE_2	5-HETE	15-HETE	LTB_4	LTC_4
Undifferentiated	–	ND	3.2	0.3	2.4	0.7
	+	ND	4.0	0.7	3.6	1.0
Differentiated	–	28	11	1.7	6.9	0.6
	+	47	25	3.1	15	2.3

Table 2. Phospholipase activities in HL-60 cells

The data shown represent activities measured in total sonicated-cell preparations, and are means ± S.D. of three separate experiments, each determined in duplicate.

Cells	Phospholipase A_2 (pmol/min per mg)	Lysophospholipase (nmol/min per mg)
Undifferentiated	5±2	0.35±0.08
Differentiated	22±6	1.04±0.3

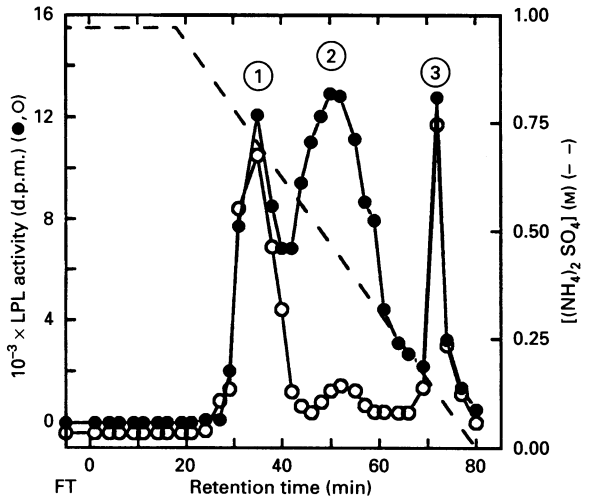

Fig. 2. Hydrophobic chromatography of HL-60 lysophospholipases

Sonicated preparations from 5.0×10^{10} undifferentiated (○) or differentiated (●) cells were applied to an Ether hydrophobic interactive column in high salt, then eluted by decreasing the salt concentration. Fractions were collected and assayed for lysophospholipase activity. In this experiment there was a 2.4-fold increase in total lysophospholipase activity in the differentiated-cell sonicated preparations. Peaks 1, 2 and 3 are indicated. In the differentiated-cell sonicated preparations, peak 2 contained approx. 60% of the total lysophospholipase activity, whereas in undifferentiated cells, peak 2 contained only about 7% of the lysophospholipase activity.

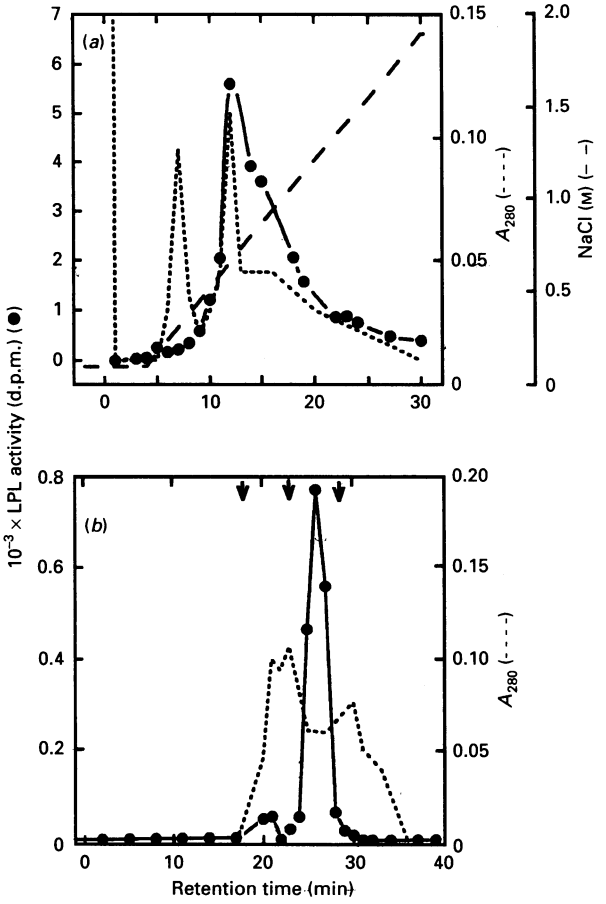

Fig. 3. Anion-exchange and gel-filtration chromatography of peak-2 lysophospholipase

The activity eluted from the hydrophobic column in fractions 42–59 was pooled, dialysed, and then applied to a strong anion-exchange column (*a*). The lysophospholipase activity was eluted from the anion-exchange column in fractions 12–20 and was concentrated and further purified on a gel-filtration column (*b*). Fractions 25–27 were pooled, concentrated by ultrafiltration and used for all subsequent studies. In (*b*) are shown the relative positions of molecular-mass standards used to calibrate the column (ovalbumin, 43 kDa; chymotrypsinogen A, 25 kDa; RNAase A, 13.7 kDa).

was contained in peak 2, and the total amount of enzyme activity contained in peaks 1 and 3 remained relatively unchanged.

Peak 2 lysophospholipase was purified by sequential anion exchange (Fig. 3*a*) and gel-filtration chromatography (Fig. 3*b*). Peak 2 was enriched by over 13000-fold (Table 3). The enzyme activity in Peak 3 from the hydrophobic column was also purified by anion-exchange (Fig. 4*a*) and gel-filtration chromatography (Fig. 4*b*). The peak 3 enzyme was enriched by over 20000-fold (Table 3). The total enrichment of peak 1 after this purification procedure was 7900-fold (result not shown). Purity of the isolated lysophospholipase was assessed by SDS/PAGE (Fig. 5). Peaks 2 and 3 had molecular masses of 20 kDa and 22 kDa respectively. The major protein constituent found in peak 1 had

Table 3. Summary of purification of HL-60-cell lysophospholipases

Data are for one preparation of differentiated cells and are representative of four independent preparations.

	Total protein (mg)	Specific activity (nmol/h per mg)	Total activity (nmol/h)	Enrichment (fold)	Total enrichment (fold)	Yield (%)
Starting material	5500	6.25	3.3×10^4			
$(NH_4)_2SO_4$ supernatant	1652	10.9	1.8×10^4	1.7		52
Ether column						
Peak 1	36	8.3×10^1	2.9×10^3	7.6	13.3	8.5
Peak 2	13.1	5.5×10^2	7.2×10^3	51	89	21
Peak 3	2.2	8.1×10^2	2.4×10^2	75	131	5
Anion-exchange						
Peak 1	0.56	7.9×10^2	4.4×10^2	10	133	1.3
Peak 2	0.48	9.6×10^2	4.6×10^2	1.7	154	1.3
Peak 3	0.075	3.1×10^3	2.4×10^2	3.9	509	0.7
Gel filtration						
Peak 1	0.0015	4.8×10^4	7.2×10^1	60	7966	0.2
Peak 2	0.00075	8.3×10^4	6.3×10^1	87	13356	0.2
Peak 3	0.00075	1.4×10^5	1.0×10^2	44	22394	0.3

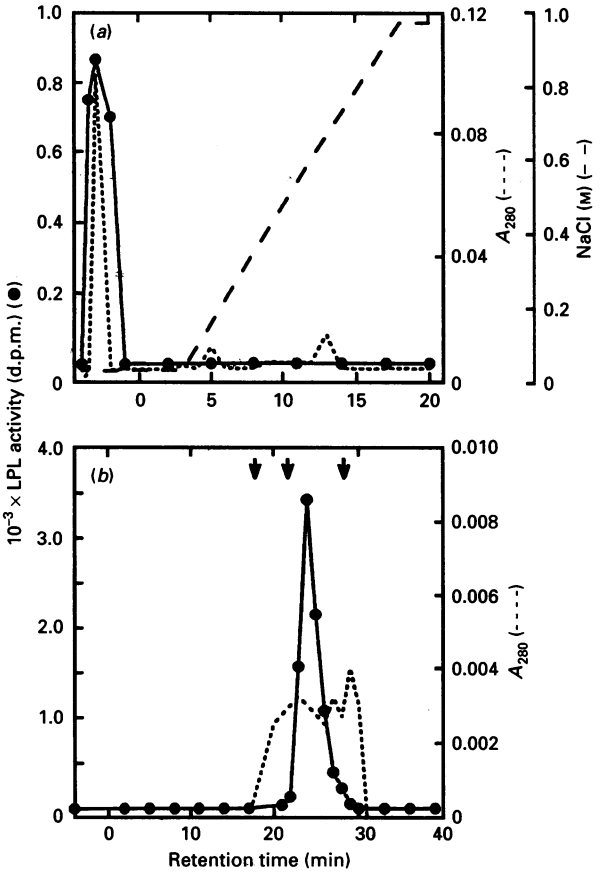

Fig. 4. Anion-exchange and gel filtration of peak 3 lysophospholipase

The lysophospholipase activity eluted from the hydrophobic column in fractions 71 and 72 was pooled, dialysed, and then applied to a strong anion-exchange column (*a*). The lysophospholipase activity failed to bind to the anion-exchange column under these conditions. The flow-through activity was pooled, concentrated by ultrafiltration and applied to a gel-filtration column (*b*). Fractions 23–26 were pooled, concentrated by ultrafiltration and used in all subsequent studies. The arrows in (*b*) represent the relative positions of molecular-mass standards used to calibrate the column (ovalbumin, 43 kDa; chymotrypsinogen A, 25 kDa; RNAase A, 13.7 kDa).

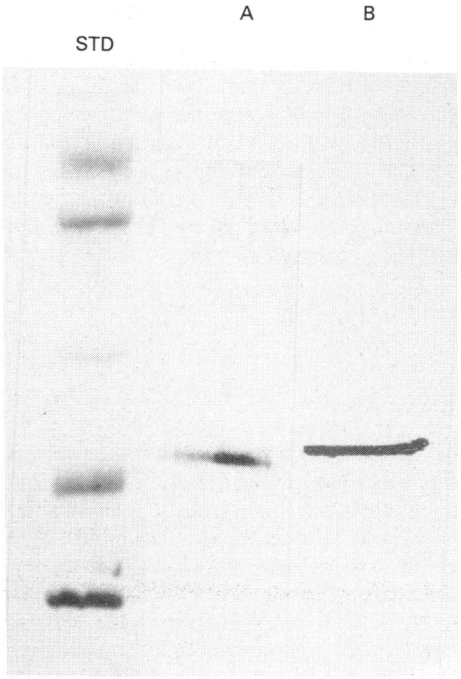

Fig. 5. SDS/PAGE of purified peak-2 and -3 lysophospholipase

Each purified lysophospholipase obtained after gel-filtration chromatography was electrophoresed on SDS/20% polyacrylamide gels. The proteins were made visible by silver staining: lane A, peak 2 lysophospholipase; lane B, peak 3 lysophospholipase. The molecular-mass standards (STD) used were 67, 43, 20 and 14 kDa.

a molecular mass of 69 kDa; however, this activity was not purified to homogeneity, and thus characterization of peak 1 lysophospholipase was not continued (results not shown).

To explore further the similarity of those two enzymes, amino acid analyses of peaks 2 and 3 were performed (Table 4). Results from these experiments indicated that these proteins had similar but distinctive amino acid compositions. Peak 3 appeared to contain additional tyrosine and isoleucine residues as compared with peak 2, which was consistent with their behaviour on the hydrophobic column. When the amino acid compositions of the

Table 4. Amino acid compositions of several lysophospholipases

*All values are expressed as mol %. References: [a] Weller *et al.* [14]; [b] Han *et al.* [33]; [c] Garsetti *et al.* [17].

Tissue source...	HL-60 peak 2	HL-60 peak 3	Human eosinophil[a]	Rat pancreatic[b]	WEHI 265.1 peak 1[c]	WEHI 265.1 peak 2[c]
Mass (kDa)...	20	21	17	67	28	27
Asx	11.1	8.3	8.0	11.6	6.9	8.4
Glx	10.5	8.6	15.2	10.5	8.0	8.6
Ser	6.6	6.3	8.6	8.0	6.1	5.6
Gly	8.5	7.7	5.0	5.0	22.4	16.9
His	2.7	4.3	2.2	2.0	11.0	7.5
Arg	4.0	4.1	5.5	3.9	1.5	3.4
Thr	5.3	5.5	4.5	5.5	5.4	4.4
Ala	7.5	7.3	4.5	4.4	9.8	8.3
Pro	8.7	8.5	5.0	6.0	0	0
Tyr	3.2	5.6	3.7	3.0	3.4	2.9
Val	7.8	8.4	9.6	7.3	4.0	7.5
Met	1.9	2.3	3.6	3.6	2.8	3.3
Ile	4.5	5.3	3.1	4.6	5.2	8.9
Leu	8.6	7.9	8.0	8.9	6.3	9.1
Phe	4.3	4.8	6.3	6.8	5.3	2.5
Lys	4.8	5.1	7.1	7.4	2.4	2.9

HL-60-cell lysophospholipases were compared with the amino acid compositions of other known lysophospholipases, there was little similarity except for pancreatic lysophospholipase, which is approx. 3 times larger in mass [12]. The *N*-termini of both proteins were blocked; therefore the *C*-terminal peptides were isolated by column chromatography using immobilized anhydrotrypsin [24]. The sequences of the *C*-terminal tryptic peptides from each protein were determined by Edman degradation and are shown below. These data indicate that peak 2 and 3 enzymes have similar, but not identical, amino acid sequences.

Peak 2 LASISLPTSNANAYFQSLIK
Peak 3 LASISLYNSNTLSYFQSLIK

DISCUSSION

Lymphocytic cells play a major role in the propagation of the inflammatory response. These cells possess phospholipase A_2 activity, which releases arachidonic acid from the phospholipids, and allows for the production of eicosanoids by both cyclooxygenase and lipoxygenase pathways (for review see [25]). Concomitant with phospholipase A_2 cleavage of the *sn*-2 arachidonic acid from phospholipid is the production of an equimolar amount of lysophospholipid. Lysophospholipids are biologically active and have been shown to be chemotactic [26], to regulate vascular permeability [27] and possibly to increase phagocytosis [28]. Furthermore, these amphipathic lysophospholipids are detergent-like molecules and are cytotoxic at high concentrations [10]. Therefore, cells have evolved mechanisms for metabolizing the lysosphospholipids produced as a consequence of phospholipase A_2 activation, and thereby attenuate the signal-transducing properties of the lysophospholipids and simultaneously protect the cell from the cytotoxic effects of lysophospholipids.

Previous reports from other laboratories, and our own, have shown that there are several different lysophospholipases [11–17]. Some lysophospholipases, such as that produced by the pancreas, are secreted into the digestive tract and appear to play a role in digestion [28]. Other lysosphospholipases, such as the eosinophil lysophospholipase, are released into the extracellular milieu and have been postulated to protect the eosinophil from the high concentration of lysophospholipid found in the surface membranes of parasites [14,29]. Other lysophospholipases have additional enzymic activites such as phospholipase A_2 or acyltransferase activity [11–13,18,30–32]. Many of the lysophospholipases appear to be different proteins, as suggested by their structural differences. For example the lysophospholipase/phospholipase A_2 protein has an apparent molecular mass of 110 kDa [18], that of pancreatic lysophospholipase is approx. 67 kDa [28], and the eosinophil lysophospholipase appears to be 15 kDa and is the smallest lysophospholipase described to date [14]. Thus, it would appear as if several different gene products have lysophospholipase activity.

Elucidation of the functions of the various lysophospholipases is complicated by the observations that even a single cell line contains multiple lysophospholipase proteins. As first described by Dennis and co-workers [15,16], the murine macrophage cell line $P388D_1$ has two different lysophospholipases, with masses of 28 and 27 kDa. Although these proteins were similar in size, they were not immunologically cross-reactive [15]. In a second murine macrophage cell line, WEHI 265.1, we demonstrated that these two lysophospholipases had different amino acid compositions, and in addition these cells also had a third lysophospholipase, which had phospholipase A_2 activity [17]. This latter protein appeared to be identical with a lysophospholipase/phospholipase A_2 that has been described by Leslie [18] in a RAW 247 mouse macrophage cell line. The functions and subcellular localization of the various lysophospholipases are not yet known.

The HL-60 cell line has been used extensively as a model system for studying the regulation of granulocyte differentiation and cellular metabolism. Inducing the differentiation of HL-60 cells with butyric acid produces a 3–4-fold increase in phospholipase A_2 enzymic activity and an increased ability of the cells to generate both cyclo-oxygenase and lipoxygenase products. We have also demonstrated that differentiated HL-60 cells have about 2–3 times more lysophospholipase activity than do undifferentiated cells. The results shown in Table 1 are similar to results obtained by previous investigators [3–8]. The increased phospolipase A_2 activity could potentially cause an increase in the levels of lysophospholipids which, if high enough, could be cytotoxic.

We have characterized the various lysophospholipases in the HL-60 cells and have determined that one of these enzymes appears to be regulated as a result of cellular differentiation. Fractionation of HL-60 cells by hydrophobic interactive chromatography resolved the total lysophospholipase activity into three peaks that were found in both differentiated and undifferentiated cells. However, the differentiated cells contain a dramatic increase in what we have termed peak 2 lysophospholipase activity. The peak 2 enzyme was very similar to the peak 3 enzyme in terms of its chromatographic properties and molecular mass. Amino acid analyses of peaks 2 and 3 demonstrated only slight differences in amino acid composition. Thus HL-60 cells, like the mouse macrophage cell lines $P388D_1$ and WEHI 265.1, have two very similar lysophospholipases. Comparison of the amino acid compositions of peaks 2 and 3 lysophospholipases with several previously described lysophospholipases (Table 4) suggests that many of the lysophospholipases are distinct polypeptides and probably have different functions and regulatory mechanisms.

Because the peak 2 lysophospholipase, which was induced in differentiated HL-60 cells, and the peak 3 enzyme appeared to be similar in molecular mass and amino acid composition, they were subjected to further structural studies. Sequence analysis of the *C*-terminal tryptic peptides of the peak 2 and 3 enzymes indicated highly similar amino acid sequences, in which only five out of 20 amino acids were different. Similar results were found upon sequencing another internal tryptic peptide from both proteins. These data suggest that both proteins are different gene products and may have evolved from a common progenitor protein. However, only peak 2 lysophospholipase appears to be induced by differentiation in these cells.

The murine macrophage cell lines characterized to date also have two similar lysophospholipase proteins. These cells do not appear to differentiate, and thus the relative functions of the two lysophospholipases is not clear. It is also interesting that the enzymes isolated from the human HL-60 cells were about 10 kDa smaller that these macrophage lysophospholipases from murine cells. Whether this is due to the difference in species (human versus mouse) or cell type (granulocyte versus macrophage) is not known. We hypothesize that the peak 2 lysophospholipase in HL-60 cells may be involved in protecting these cells from the cytolytic effects of the lysophospholipids produced as a consequence of phospholipase A_2 catalysis; however; the roles of the other lysophospholipases in these cells at present remain unknown.

REFERENCES

1. Collins, S. J., Ruscetti, F. W., Gallagher, R. E. & Gallo, R. C. (1978) Proc. Natl. Acad. Sci. U.S.A. **75**, 2458–2462
2. Rovera, G., Santoli, D. & Damski, C. (1979) Proc. Natl. Acad. Sci. U.S.A. **76**, 2779–2782

3. Hoessly, M. C., Rossi, R. M. & Fischkoff, S. A. (1989) Cancer Res. **49**, 3594–3597
4. Agins, A. P., Zipkin, R. E. & Taffer, I. M. (1989) Agents Actions **21**, 397–399
5. Anthes, J. C., Bryant, R. W., Musch, M. W., Ng, K. & Siegal, M. I. (1986) Inflammation **10**, 145–156
6. Goerig, M., Habenicht, A. J. R., Zeh, W., Salbach, P., Kommerell, B., Roth, D. E. R., Nastainczyk, W. & Glonset, J. A. (1988) J. Biol. Chem. **263**, 19384–19391
7. Ziboh, V. A., Wong, T., Wu, M. C. & Yunis, A. A.. (1986) J. Lab. Clin. Med. **108**, 161–166
8. Flower, R. J. & Blackwell, G. J. (1976) Biochem. Pharmacol. **25**, 285–291
9. Oishi, K., Raynor, R. L., Charp, R. A. & Kuo, J. F. (1988) J. Biol. Chem. **263**, 6865–6871
10. Welthzien, H. U. (1979) Biochim. Biophys. Acta **559**, 259–287
11. Gross, R. W. & Sobel, B. E. (1982) J. Biol. Chem. **257**, 6702–6708
12. De Jong, J. G. N., Van Den Bosch, H., Aarsman, A. J. & Van Deenen, L. L. M. (1973) Biochim. Biophys. Acta **296**, 105–115
13. De Jong, J. G. N., Van Den Bosch, H., Rijken, D. & Van Deenen, L. L. M. (1974) Biochim. Biophys. Acta **369**, 50–63
14. Weller, P. F., Bach, D. S. & Austen, K. F. (1984) J. Biol. Chem. **259**, 15100–15106
15. Zhang, Y. Y., Deems, R. A. & Dennis, E. A.. (1991) Methods Enzymol. **197**, 456–468
16. Zhang, Y. & Dennis, E. A. (1988) J. Biol. Chem. **263**, 9965–9972
17. Garsetti, D. E., Ozgur, L. E., Steiner,, M. R., Egan, R. W. & Clark, M. A. (1992) Biochim. Biophys. Acta, in the press
18. Leslie, C. C. (1991) J. Biol. Chem. **266**, 11366–11371
19. Fischkoff, S. A., Brown, G. E. & Pollack, A. (1986) Blood **68**, 185–192
20. Bradford, M. M. (1976) Anal. Biochem. **72**, 248–254
21. Clark, M. A., Littejohn, D., Conway, T. M., Mong, S., Steiner, S. & Crooke, S. T. (1986) J. Biol. Chem. **261**, 10713–10718
22. Radin, N. S. (1967) Methods Enzymol. **14**, 245–255
23. Simpson, R. J., Mortz, R. L., Begg, G. S., Rubina, M. R. & Nice, E. C. (1989) Anal. Biochem. **177**, 221–235
24. Arisaka, F., Nakako, T., Kumazaki, T. & Ishii, S. (1987) J. Protein Chem. **6**, 245–251
25. Dennis, E. A. (1987) Biotechnology **5**, 1294–1299
26. Quinn, M. T., Parthasarathy, S. & Steinberg, D. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 2805–2809
27. Kugiyama, K., Kerns, S. A., Morrisett, J. D., Roberts, R. & Herery, P. D. (1990) Nature (London) **344**, 160–162
28. Elsbach, P., Van den Berg, J. W. O. & Van Deenen, L. L. M. (1965) Biochim. Biophys. Acta **106**, 338–343
29. Golan, M. E., Brown, C. S., Cianci, M. L., Furlong, S. T. & Caulfield, J. P. (1986) J. Cell Biol. **103**, 819–828
30. Gross, R. W., Drisdel, R. C. & Sobel., B. E. (1983) J. Biol. Chem. **258**, 15165–15172
31. Kramer, R. M., Roberts, E. F., Manetta, J. & Putnam, J. E. (1991) J. Biol. Chem. **266**, 5268–5272
32. Clark, J. D., Milona, N. & Knopf, J. L. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 7708–7712
33. Han, J. H., Stratowa, C. & Rutter, W. J. (1987) Biochemistry **20**, 1617–1625

Received 2 April 1992/15 June 1992; accepted 3 July 1992