Characterization and purification of a lipoxygenase inhibitor in human epidermoid carcinoma A431 cells

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A lipoxygenase inhibitor in the cytosolic fraction of human epidermoid carcinoma A431 cells was characterized and purified. The cytosolic inhibitor lost the inhibitory activity upon heating at 75 °C for 15 min or pretreating with 1 mg/ml trypsin at 37 °C for 60 min. Cytosol, after dialysis, lost the inhibitory activity but its inhibitory activity recovered when 1 mM GSH was added to the dialysate. The inhibitory activity of cytosol was also abolished by treatment either with 1 mM iodoacetate at 4 °C for 1 h or with 0.5 mM H_2O_2. The pI of the inhibitor was approx. 7.0. In addition to 12-lipoxygenase, the inhibitor inhibited the activities of 5-lipoxygenase and fatty acid cyclo-oxygenase in a cell-free system. The inhibitor was purified by a series of column chromatographies using CM Sephadex C-50, Sephadex G-100 SF and Mono P columns. A major 22 kDa protein was obtained that was distinct from selenium-dependent glutathione peroxidase.

INTRODUCTION

Oxidative stress is associated with a disturbance in the reactive oxygen species/antioxidant balance in favour of the former [1]. Many of the reactive oxygen species are oxygen free radicals, which are involved in oxidative damage in a number of physiological and pathophysiological phenomena, and processes as diverse as inflammation, aging, carcinogenesis, drug action and drug toxicity. Reactive oxygen species may be formed from polyunsaturated fatty acids either by enzymic or by non-enzymic oxygenation. Reduction of the reactive oxygen species can be achieved either by the inhibition of lipid oxygenation enzymes or by the interception of free radicals by antioxidants.

Lipoxygenases and fatty acid cyclo-oxygenase are the major enzymes involved in the metabolism of polyunsaturated fatty acids, including arachidonic acid [2]. Arachidonic acid is metabolized to 5-, 12- and 15-hydroperoxyeicosatetraenoic acids by 5-, 12- and 15-lipoxygenases respectively. These hydroperoxides belong to one group of reactive oxygen species, and are metabolized to hydroxide compounds usually by a glutathione-dependent peroxidase system [3]. Arachidonic acid is also metabolized to prostaglandin (PG)G_2 and PGH_2 by fatty acid cyclo-oxygenase, followed by the formation of PGE_2, $PGF_2\alpha$, thromboxanes and other PGs.

In studying the regulation of arachidonate metabolism in human epidermoid carcinoma A431 cells, we previously showed that epidermal growth factor (EGF) induced the expression of human platelet-type 12-lipoxygenase in levels of enzyme protein and mRNA [4–6]. A putative inhibitor of 12-lipoxygenase was detected in cytosol, which inhibited the biosynthesis of 12(*S*)-hydroxyeicosatetraenoic acid [12(*S*)-HETE] by the microsomal 12-lipoxygenase in a cell-free system, and masked 12(*S*)-HETE biosynthesis in intact cells [4]. In the present study, we characterized this inhibitor in A431 cells and achieved its purification by column chromatographies. The results indicate that the inhibitor was regulated by the cellular reduction–oxidation (redox) conditions. The successful purification of the inhibitor provides several leads for the study of the cellular regulation of lipid hydroperoxide formation by the arachidonate lipoxygenases.

MATERIALS AND METHODS

Materials

Mouse EGF (natural, culture grade) was purchased from Collaborative Research (Bedford, MA, U.S.A.). [1-^{14}C]Arachidonic acid (58.0 mCi/mmol) was purchased from Amersham. Low-molecular-mass standards for gel electrophoresis and dye reagent concentrate for protein assay were from Bio-Rad Laboratories (Hercules, CA, U.S.A.). Iodoacetate, 1,4-dithiothreitol (DTT), soya-bean trypsin inhibitor and silica gel 60 TLC plates (0.25 mm thickness) were from E. Merck AG (Darmstadt, Germany). Reduced glutathione (GSH) was from Boehringer Mannheim (Mannheim, Germany). PGE_2, 15-keto PGE_2, haemoglobin, adrenaline (epinephrine) and BSA (fraction V) were from Sigma (St. Louis, MO, U.S.A.). Trypsin was from Difco Laboratories (Detroit, MI, U.S.A.). Sephadex G-100, Sephadex G-100 SF (superfine), CM-Sephadex C-50, Mono P (HR 5/20) and Polybuffer 96 were from Pharmacia (Uppsala, Sweden). Disc membrane YM10 was from Amicon (Beverly, MA, U.S.A.) and Centrisart I (10 kDa cut-off filter) was from Sartorius (Goettingen, Germany). Dulbecco's modified Eagle's medium was obtained from Gibco BRL (Grand Island, NY, U.S.A.), and fetal-bovine serum was from Hyclone Laboratories (Logan, UT, U.S.A.).

Cell culture

Human epidermoid carcinoma A431 cells were grown at 37 °C under air/CO_2 (19:1) in 10-cm plastic dishes containing 10 ml of culture medium consisting of Dulbecco's modified Eagle's medium supplemented with 10% (v/v) fetal-bovine serum, 100 μg/ml streptomycin and 100 units/ml penicillin. The confluent cells were subcultured with a ratio of 1:4. On the 5th

Abbreviations used: PG, prostaglandin; EGF, epidermal growth factor; HETE, hydroxyeicosatetraenoic acid; DTT, 1,4-dithiothreitol; RBL, rat basophilic leukaemia.

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day after subculture, the confluent cells were treated with 50 ng/ml EGF for 18 h to induce the expression of 12-lipoxygenase and fatty acid cyclo-oxygenase for use in this series of experiments. The same culture medium was also used in the suspension culture of rat basophilic leukaemia (RBL-1) cells.

Preparation of microsomes and cytosol

A431 cells in a 10-cm Petri dish were washed twice with PBS and scraped with a Teflon sheet in 0.5 ml of 50 mM Tris/HCl, pH 7.4, unless stated otherwise, before being subjected to sonication. The homogenate was centrifuged at 9000 ***g*** for 20 min and the resulting supernatant was recentrifuged at 105000 ***g*** for 1 h in a Beckman L8-80M ultracentrifuge. The resulting pellet was resuspended in 0.5 ml of 50 mM Tris/HCl, pH 7.4, as the microsomal fraction, and the supernatant was designated as the cytosolic fraction.

Enzyme activity assay

The assay mixture contained 8.5 μM [1-^{14}C]arachidonic acid (0.1 μCi) and an appropriate amount of protein in microsomes and cytosol in a final volume of 0.2 ml. The reaction was allowed to take place at 37 °C for 20 min. The reaction mixture was acidified to pH 3.0 with 1 M HCl, extracted with 2 ml of ethyl acetate, and evaporated with N_2 gas. The residues were dissolved in ethanol and applied to TLC plates, together with eicosanoid standards if necessary. The plates were developed in the organic phase of a solvent system of ethyl acetate/2,2,4-trimethylpentane/acetic acid/water (11:5:2:10, by vol.). Product formation was determined by a System 2000 Imaging Scanner (BIOSCAN).

Purification procedure

Cytosol of A431 cells was prepared with 50 mM ammonium acetate buffer containing 0.5 mM EDTA and 1 mM DTT (Buffer A), pH 6. It was applied to a CM-Sephadex C-50 column (2.4 cm × 18 cm) and washed with Buffer A, pH 6. The proteins were eluted with a salt gradient formed with 200 ml of Buffer A, pH 6, and Buffer B composed of 1 M ammonium acetate, 0.5 mM EDTA and 1 mM DTT, pH 8. Fractions having the inhibitor activity were concentrated with a YM10 membrane, and applied to a Sephadex G-100 SF column (2 cm × 55 cm). The proteins were eluted with Buffer A, pH 7.4. The inhibitor fraction was concentrated and applied to a Mono P column (HR5/20) on a FPLC system (Pharmacia). The column was first equilibrated with 50 mM ammonium acetate buffer containing 1 mM DTT, pH 8, and 1:20 dilution of Polybuffer 96 in the presence of 1 mM DTT at pH 5.3 was then used for the elution of the inhibitor. One unit of the activity of inhibitor was defined as a 50% inhibition of 12(*S*)-HETE formation generated by 0.15 mg of microsomes.

Protein determination

Protein content of all samples except the eluate of Mono P column chromatography was determined by the method of Lowry et al. [7], and that of the eluate from FPLC by a Bio-Rad protein assay system with BSA (fraction V) as a standard.

SDS/PAGE

An analytical SDS/PAGE (14% (w/v) gel) was performed by the method of Laemmli [8]. The proteins on gel were detected by Bio-Rad silver stain by the method of Merrill et al. [9], and the density was determined by an image analysis system installed with the software BIO-ID (Vilber Lourmat, Marne la Vallée, France).

RESULTS

Characteristics of the inhibitor

The activity of inhibitor was completely lost when cytosol was heated at 75 °C for 15 min. Treatment of cytosol with 1 mg/ml trypsin at 37 °C for 1 h, which was stopped by the addition of 1 mg/ml soya-bean trypsin inhibitor, completely eliminated inhibitory activity (results not shown). The approximate molecular mass of the inhibitor in cytosol was determined by the molecular-mass cut-off filter and gel filtration. The inhibitor did not go through the filter, which cuts off molecules with molecular mass 10 kDa. Sephadex G-100 column chromatography placed the molecular mass of the inhibitor in crude cytosol at approx. 15–22 kDa (results not shown). In order to study the possibility of binding of inhibitor to the substrate, the enzyme activity assay mixture was analysed by Sephadex G-25 column chromatography, and no substrate binding was observed (results not shown).

Modification of the stability of the inhibitor by redox reaction

The activity of the inhibitor in cytosol was potentiated when cytosol was prepared in the presence of 1 mM GSH (Figure 1). As indicated in Table 1, the activity of 12-lipoxygenase was

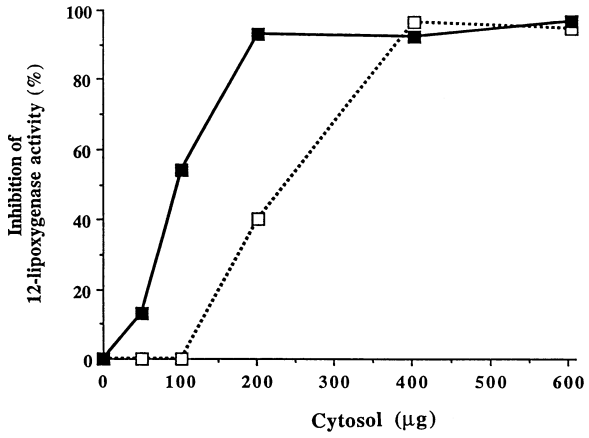

Figure 1 Effect of glutathione on the activity of the inhibitor

The microsomal fraction (0.37 mg of protein) was incubated with [1-^{14}C]arachidonic acid in the presence of cytosol, which was prepared in the absence (□) or presence (■) of 1 mM GSH.

Table 1 Effect of dialysis on the activity of the inhibitor

Cytosol prepared from A431 cells was dialysed against 50 mM Tris/HCl buffer, pH 7.4, overnight. The activity of the inhibitor in cytosol and dialysed cytosol was determined. In the enzyme activity assay, 0.22 mg of microsomes and 0.53 mg of cytosol were used. Values are means ± S.E.M. of three determinations.

	Presence of microsomes	$10^3 \times 12(S)$-HETE (c.p.m./mg of protein)
None	+	37 ± 0.97
Cytosol	+	13 ± 2.4
Dialysed cytosol	+	77 ± 1.1
Dialysed cytosol	−	36 ± 0.56

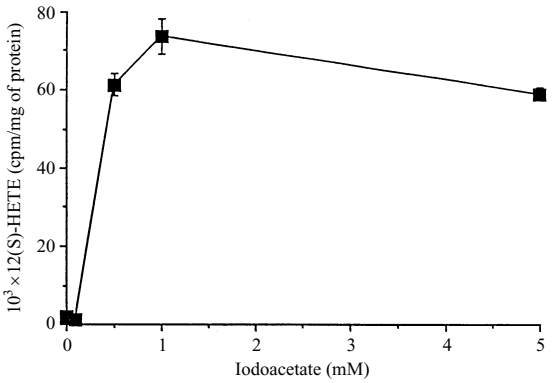

Figure 2 Effect of iodoacetate on the activity of the inhibitor

Cytosol (2.1 mg of protein) was treated with various doses of iodoacetate at 4 °C for 1 h, and then incubated with [1-^{14}C]arachidonic acid for the 12-lipoxygenase activity assay. Values are means ± S.E.M. of three determinations.

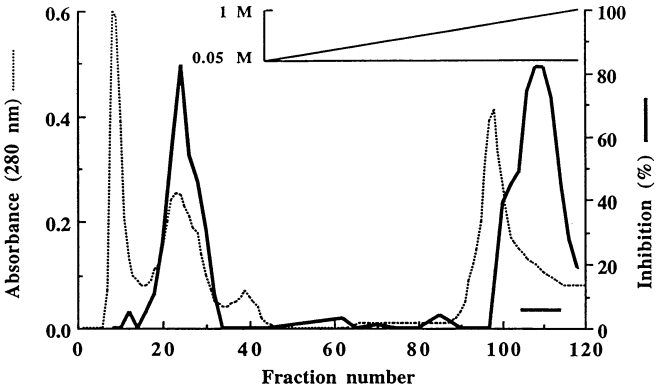

Figure 3 CM-Sephadex C-50 column chromatography

Cytosol (25 mg of protein) was applied to a column (2.4 cm × 18 cm) of CM-Sephadex C-50 equilibrated with running buffer (50 mM ammonium acetate/1 mM DTT/0.5 mM EDTA, pH 6). Proteins were then eluted with a linear gradient buffer composed of ammonium acetate from 0.05 to 1 M (see inset). The flow rate was 2.2 ml/10 min. Each fraction, with a volume of 2.2 ml, was collected, and 50 μl of each fraction was used in the enzyme activity assay. Fractions numbered from 105 to 114 were pooled for the next step of purification.

measurable in the dialysed cytosol due to inactivation of the inhibitor in dialysis, indicating the presence of 12-lipoxygenase in the cytosol. The activity of the inhibitor in the dialysed cytosol was completely recovered by the addition of 1 mM GSH (results not shown). The activity of the inhibitor in the cytosol was blocked by treating the cytosol with iodoacetate in a dose-dependent manner, whose maximum effect was reached at a concentration of 1 mM (Figure 2). The activity of partially purified inhibitor obtained from CM-Sephadex C-50 and Sephadex G-100 SF column chromatographies was also blocked by iodoacetate in a dose-dependent manner (results not show). The inhibitor was also sensitive to H_2O_2. Treatment of cytosol with H_2O_2 at 0.05, 0.5 and 2 mM resulted in 10%, 90% and 95% inactivation of the cytosolic inhibitor respectively.

Purification of inhibitor

In the CM-Sephadex C-50 column chromatography, the inhibitor was eluted by a linear gradient buffer with ammonium acetate from 0.05 to 1 M (Figure 3). Fractions having the inhibitor activity were collected and concentrated. The concentrate pooled from several experiments from CM-Sephadex C-50 column chromatography was applied to Sephadex G-100 SF column chromatography, and the inhibitor was eluted with 50 mM ammonium acetate buffer containing 1 mM DTT and 0.5 mM EDTA, pH 7.4 (Figure 4). Fractions having the inhibitor activity were collected and concentrated. The concentrate was applied to Mono P column chromatography, and the column was eluted with Polybuffer 96 at a pH gradient of 8.0–5.3. As shown in Figure 5(A), the inhibitor was eluted at pH 7. The density of the major 22 kDa protein correlated well with the activity of the inhibitor (Figures 5B and 5C). The pattern of SDS/PAGE in each purification step is shown is Figure 6, and the purification of the inhibitor in this series of chromatographies is summarized in Table 2. A 2144-fold purification with a 6% yield was achieved.

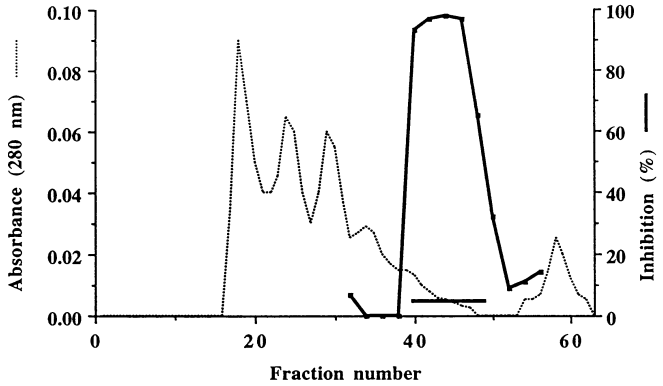

Figure 4 Sephadex G-100 SF column chromatography

The concentrated sample (1.25 mg of protein) was applied to a column (2 cm × 55 cm) of Sephadex G-100 SF equilibrated with running buffer (50 mM ammonium acetate/1 mM DTT/0.5 mM EDTA, pH 7.4). The proteins were eluted with the same buffer. The flow rate was 2 ml/30 min. Each fraction, with a volume of 2 ml, was collected, and 50 μl of each fraction was used in the enzyme activity assay. Fractions numbered from 39 to 49 were pooled for the next step of purification.

Specificity of inhibitor

The partially purified inhibitor obtained from CM-Sephadex C-50 and Sephadex G-100 SF column chromatographies was used in this series of experiments. The inhibitory effect of inhibitor on 5-lipoxygenase in cytosol of RBL-1 cells was compared with that on 12-lipoxygenase in microsomes of A431 cells. RBL-1 cytosol (40 μg of protein) and A431 microsomes (41 μg of protein) were used in enzyme activity assays, because at these concentrations they produced the same amount of 5-hydroxyeicosatetraenoic acid (5-HETE) and 12(*S*)-HETE from arachidonic acid respectively. As shown in Figure 7, 5-lipoxygenase was also inhibited by the inhibitor, but it was less sensitive to inhibitor than 12-lipoxygenase. The effect of the inhibitor on the activity of fatty acid cyclo-oxygenase in cell-free system was then studied. The inhibitor inhibited the biosynthesis of both PGE_2 and 12(*S*)-HETE in the microsomes of A431 cells in a similar dose-dependent manner (results not shown). The inhibitory effect of inhibitor was also confirmed in the crude cell homogenate. Comparing the low biosynthesis of 12(*S*)-HETE, PGE_2 and 15-keto PGE_2 in control cell homogenate, a significant biosynthesis of 12(*S*)-HETE, PGE_2 and 15-keto PGE_2 was observed in

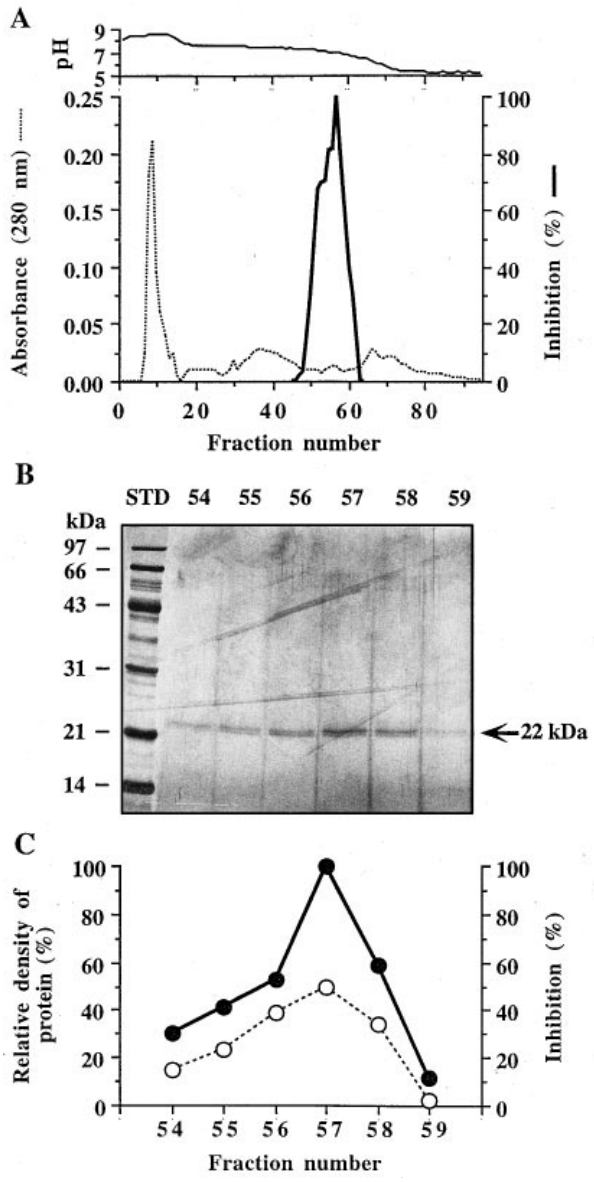

Figure 5 Mono P column chromatography

(**A**) The concentrated sample (0.866 mg of protein) was applied to a column (HR 5/20) of Mono P equilibrated with running buffer (50 mM ammonium acetate/1 mM DTT, pH 8). The proteins were eluted with the elution buffer (Polybuffer 96 with 1:20 dilution in the presence of 1 mM DTT, pH 5.3). The flow rate was 30 ml/60 min. Each fraction, with a volume of 0.5 ml, was collected, and 10 μl of each fraction was used in the enzyme activity assay. (**B**) Fractions numbered from 54 to 59 (25 μl of each) were analysed by SDS/PAGE (14% gels) analysis, and proteins were detected by silver stain. The molecular mass of the inhibitor was calculated from the mobility of standard proteins (STD), the molecular masses of which are given on the left. The position of the 22 kDa protein is shown on the right. (**C**) Relative density of the 22 kDa protein (●) and the percentage of inhibition (○) in fractions 54–59 were compared. In the enzyme activity assay, 1 μl of each fraction was used.

iodoacetate-treated cell homogenate, in which the inhibitor was inactivated (Table 3).

DISCUSSION

A heat-labile GSH-dependent cytosolic factor in rat liver [10–13] and heart [14] inhibits the peroxidative attack on polyunsaturated fatty acids in the membrane lipids, which is generated

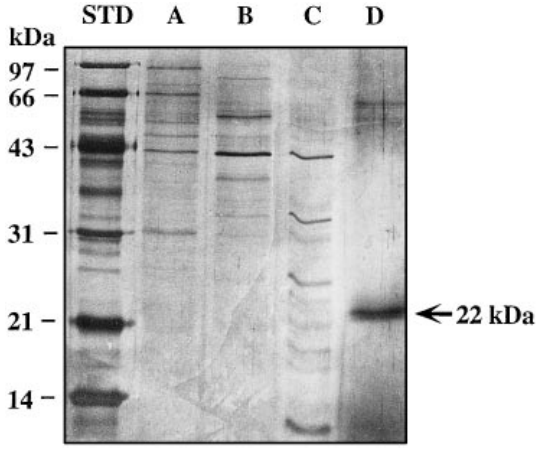

Figure 6 Purity analysis of inhibitor

Samples (100 ng of protein for each) with inhibitor activity from: lane A, cytosol; lane B, CM-Sephadex C-50, lane C, Sephadex G-100 SF; lane D, Mono P; column chromatographies were analysed by SDS/PAGE, and proteins were detected by silver staining. Positions of molecular mass markers are given on the left and the position of the 22 kDa protein is given on the right.

by an NADPH-generating system. One or more thiol groups associated with the factor is required for the inhibition [14,15], and the inhibitor is distinct from glutathione peroxidase and glutathione *S*-transferase [13]. However, neither its purification nor its effect on the arachidonate metabolism has been reported. Several groups of investigators have provided evidence pointing to the presence of a lipoxygenase inhibitor in the cytosolic fraction of cultured cells. Our laboratory reported an endogenous inhibitor of 12-lipoxygenase, which masked 12(*S*)-HETE biosynthesis in intact cells, in the cytosolic fraction of human epidermoid carcinoma A431 cells [4]. The inhibitory effect of the cytosolic fraction on 12-lipoxygenase activity was also observed in primary culture of ovine tracheal epithelial cells, and the putative inhibitor was inactivated by treating cytosol with a GSH-depleting agent and lipid hydroperoxide [16]. The presence of cytosolic inhibitor of 12-lipoxygenase in murine W256 cells and Lewis lung carcinoma cells has also been suggested, but remains undocumented [17].

Our results indicate that the inhibitor in the cytosol of A431 cells was heat labile, and its activity was stabilized by the presence of GSH and required thiol group. These characteristics are comparable with those of the inhibitor detected in rat liver [13]. The inhibitor in A431 cells was inactivated by H_2O_2 as was that in cultured ovine tracheal epithelial cells [16]. When dialysed, its activity disappeared, but was recoverable by the addition of 1 mM GSH to the dialysate, indicating that the inhibitor was not dialysable and that its activity was regulated by a GSH-dependent redox reaction. Taken together, the lipoxygenase inhibitor in A431 cells was quite similar to those in rat liver and ovine tracheal epithelial cells in its biochemical behaviour.

Neither the lipid oxygenation inhibitor in rat liver nor that in ovine tracheal epithelial cells has been extensively purified. Burk et al. [18] applied the $> 30\%$-satd.-$(NH_4)_2SO_4$ fraction from rat liver cytosol to Sephadex G-100 column chromatography, and determined the ability to protect against free malonaldehyde formation in the NADPH–microsomal lipid peroxidation system. They found a broad peak of oxidation-inhibitory activity in the eluates and suggested that it may have more than one component. In our hands, Sephadex G-100 chromatography also revealed a

Table 2 Purification of the lipoxygenase inhibitor

Step	Total protein (mg)	Total activity (units)	Specific activity (units/mg)	Purification (fold)	Yield (%)
Cytosol	319	14153	44	1	100
CM-Sephadex C-50	11.9	4240	355	8	30
Sephadex G-100 SF	0.92	3840	4249	95	27
Mono P	0.009	855	95000	2144	6

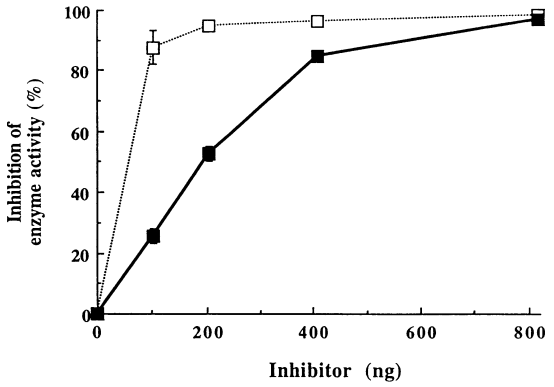

Figure 7 Effect of the inhibitor on 5- and 12-lipoxygenases

RBL-1 cytosol (0.08 mg of protein) and A431 microsomes (0.08 mg of protein) were incubated with [1-^{14}C]arachidonic acid in the presence of various doses of inhibitor obtained from CM-Sephadex C-50 and Sephadex G-100 SF column chromatography. The enzyme activity assay of 5-lipoxygenase (■) and 12-lipoxygenase (□) was performed as described in the Materials and methods section. Values are means ± S.E.M. of three determinations.

Table 3 Arachidonate metabolism in iodoacetate-treated crude cell homogenate

Cells scraped with 50 mM Tris/HCl buffer, pH 7.4, were sonicated. After centrifugation at 100 ***g*** for 10 min, the supernatant was obtained as crude homogenate. The crude homogenate was treated with 1 mM iodoacetate at 4 °C for 1 h. [1-^{14}C]Arachidonate metabolism by control and iodoacetate-treated crude homogenates (1.1 mg of protein for each) was performed by the enzyme activity assay described in the Materials and methods section, and the formation of 12(*S*)-HETE, PGE_2 and 15-keto PGE_2 was analysed. Value are means ± S.E.M. of three determinations.

Treatment	$10^3 \times$ 12(*S*)-HETE (c.p.m./mg of protein)	$10^3 \times PGE_2$ (c.p.m./mg of protein)	$10^3 \times$ 15-keto PGE_2 (c.p.m./mg of protein)
Control	0.49 ± 0.05	2.2 ± 0.11	0.23 ± 0.01
Iodoacetate	40 ± 3.3	13 ± 0.13	8.6 ± 0.57

broad peak ranging from 15 to 22 kDa of inhibitor in crude cytosol of A431 cells. Yet, whether the inhibitor consists of more than one component requires additional study. In this study, the inhibitor was purified only to 22 kDa but not beyond. The inhibitor was also distinct from selenium-dependent glutathione peroxidase, since the gel-filtration profiles were different and no glutathione peroxidase activity in the purified inhibitor was detected using cumene hydroperoxide as substrate (C.-J. Chen, H.-S. Huang, Y.-T. Lee, C.-T. Yang and W.-C. Chang, unpublished work). A 2144-fold purification was achieved (Table 2), indicating that the content of inhibitor in cells was very low.

The activity of human 12-lipoxygenase has been detected in the microsomal fraction of A431 cells but not in the cytosolic fraction [4]. In the present study, however, two pieces of evidence were obtained to indicate its presence in the cytosolic fraction. First, cytosolic 12-lipoxygenase activity was recovered if the cytosol was dialysed, and second, it was also detected if the cytosol was treated with either iodoacetate or H_2O_2. The distribution ratio of 12-lipoxygenase in the microsomes and the iodoacetate-treated cytosol of A431 cells was 2:1 [6]. It is still uncertain whether the recovery of 12-lipoxygenase in the microsomal fraction was due to binding to the membrane or to translocation during subcellular fractionation. With the aid of the immunoelectronic microscopy, Nakamura et al. [19] reported that the platelet-type 12-lipoxygenase was mainly localized in the cytoplasm in platelets and megakaryocytes.

In addition to the inhibition of 12-lipoxygenase in A431 cells, the inhibitor also inhibited the activities of 5-lipoxygenase in RBL-1 cytosol and fatty acid cyclo-oxygenase in A431 microsomes. Significant biosynthesis of 12(*S*)-HETE, PGE_2 and 15-keto PGE_2 in crude cell homogenate treated with iodoacetate provided another piece of evidence indicating that the inhibitor inhibited the enzyme activities of both 12-lipoxygenase and fatty acid cyclo-oxygenase in the cell-free system. The inhibition was not due to the substrate binding of inhibitor. It is of particular interest to rationalize the possible mechanism of the inhibition of both arachidonate lipoxygenases and fatty acid cyclo-oxygenase. Non-haem iron is an essential component of arachidonate lipoxygenases [20–23], and the haemoprotein nature of fatty acid cyclo-oxygenase is well known [24,25]. Reduction of ferric ion to the ferrous state in lipoxygenases is a critical reaction in the initiation of the formation of fatty acid free radicals [26,27], and haem in its ferryl state coupled to the tyrosyl radical in the cyclo-oxygenase reaction is involved in its formation [28,29]. Whether the inhibitor interferes with the functions of iron in lipoxygenases and fatty acid cyclo-oxygenase awaits further study. In A431 cells with EGF-induced expression of 12-lipoxygenase and fatty acid cyclo-oxygenase, which were challenged with calcium ionophore A23187, PGE_2 formation increased by 3-fold but 12(*S*)-HETE remained unchanged [4]. These results clearly indicate that 12-lipoxygenase, but not fatty acid cyclo-oxygenase, was negatively regulated by the inhibitor in intact cells. Recent immunocytochemical studies have revealed that both the fatty acid cyclo-oxygenases 1 and 2 are localized in the lumina of endoplasmic reticulum and nuclear envelope [30–33], which may prevent fatty acid cyclo-oxygenase from interacting with the cytosolic inhibitor in intact cells.

In conclusion, a heat-labile GSH-dependent inhibitor of arachidonate lipoxygenases and fatty acid cyclo-oxygenase in the soluble fraction of A431 cells was present. The inhibitor protein was purified with a molecular mass of 22 kDa. Its heat-lability and GSH-dependency resembled those demonstrated in rat liver

cytosol [13–15], but it is still not clear whether they are one and the same protein. The molecular mass of the liver factor and its effect on the arachidonate dioxygenation are still uncertain. Although the inhibitor effectively inhibited the arachidonate lipoxygenases and fatty acid cyclo-oxygenase in the cell-free system, our previous results [4] indicate that the PG biosynthesis in intact A431 cells was evidently not affected. The physiological and pathophysiological role of PGs and thromboxanes in the cell will therefore not be disturbed by the inhibitor. The presence of inhibitor in cells, which prevents the formation of lipid hydroperoxides catalysed by lipoxygenases, may play a significant role in some physiological and pathophysiological states related to the formation of reactive oxygen species.

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