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Chemical Modification of 5-Lipoxygenase from the Korean Red Potato

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The lipoxygenase was purified 35 fold to homogeneity from the Korean red potato by an ammonium sulfate precipitation and DEAE-cellulose column chromatography. The simple purification method is useful for the preparation of pure lipoxygenase. The molecular weight of the enzyme was estimated to be 38,000 by SDS-polyacrylamide gel electrophoresis and Sepharose 6B column chromatography. The purified enzyme with 2 M $(NH_4)_2SO_4$ in a potassium phosphate buffer, pH 7.0, was very stable for 5 months at -20°C. Because the purified lipoxygenase is very stable, it could be useful for the screening of a lipoxygenase inhibitor. The optimal pH and temperature for lipoxygenases purified from the red potato were found to be pH 9.0. and 30°C, respectively. The Km and Vmax values for linoleic acid of the lipoxygenase purified from the red potato were 48 μM and 0.03 μM per minute per milligram of protein, respectively. The enzyme was insensitive to the metal chelating agents tested (2 mM KCN, 1 and 10 mM EDTA, and 1 mM NaN_3), but was inhibited by several divalent cations, such as Cu^{++}, Co^{++} and Ni^{++}. The essential amino acids that were involved in the catalytic mechanism of the 5-lipoxygenase from the Korean red potato were determined by chemical modification studies. The catalytic activity of lipoxygenase from the red potato was seriously reduced after treatment with a diethylpyrocarbonate (DEPC) modifying histidine residue and Woodward's reagent (WRK) modifying aspartic/glutamic acid. The inactivation reaction of DEPC (WRK) proceeded in the form of pseudo-first-order kinetics. The double-logarithmic plot of the observed pseudo-first-order rate constant against the modifier concentration yielded a reaction order 2, indicating that two histidine residues (carboxylic acids) were essential for the lipoxygenase activity from the red potato. The linoleic acid protected the enzyme against inactivation by DEPC(WRK), revealing that histidine and carboxylic amino acids residues were present at the substrate binding site of the enzyme molecules.

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Lipoxygenases (EC 1.13.11.12) are a group of closely related enzymes that appear to be widely distributed in plants (Ohta *et al*, 1986; Todd *et al.*, 1990; Battu, 1994; Zhang and Hamberg, 1996; Butovich *et al*, 1998) and mammalian tissues (Clapp *et al.*, 1985; Rouzer *et al*, 1985; Kemal *et al.*, 1987). They catalyzed the dioxygenation of polyunsaturated fatty acids that contained all of the cis-methylene-interrupted double bonds. The nomenclature for lipoxygenases is based on the site of insertion of molecular oxygen onto the fatty acid molecule, in this case, arachidonic acid. Accordingly, they have been classified as 5-, 8-, 9-, 11-, 12- (Nakatsuka *et al.*, 1994; Huaaey and Yisdale, 1996; Yamamoto *et al*, 1997), and 15-lipoxygenase (Kemal *et al.* 1987; Nihel *et al.*, 1993) and are found in plants and animals (Skeiya and Okuda, 1982). One of the most physiologically important lipoxygenases, as well as the subject of great interest in recent years, is 5-lipoxygenase (Sun and McGuire, 1983; Schimizu *et al.*, 1986; Percival, 1991), which catalyzes the oxygenation of archidonic acid to 5-hydroperoxyeicosatetraenoic acid and its subsequent dehydration to form leukotriense A_4. Leukotriense A_4 (Samuelsson, 1983) is the substrate for the synthesis of the biologically active leukotriene LTB_4 and peptidoleukotriene LTC_4, LTD_4, and LTE_4. The leukotrienes have a wide range of biological activities (Evans *et al*, 1987; Piomelli *et al*, 1987) that included: lymphocyte chemotaxis and chemokinesis, enhancement of lymphocyte cytotoxicity, contraction of lung tissue, enhancement of vascular permeability, and mucus hypersecretion. Consequently, it is believed that leukotriens have a role in the pathophysiology of allergy reaction and inflammation (Walker *et al.* 1980; Shimizu *et al.*, 1986). Thus, 5-lipoxygenase is a drug target that has a great therapeutic significance. The 5-Lipoxygenase inhibitors are expected to be useful for the treatment of allergies and inflammations such as asthma, psoriasis and hypersensitivity. The 5-Lipoxygenase was purified from a number of mammalian cells, including human (Mylari *et al.*, 1990) and porcine leukocytes, as well as from plants (Gardner, 1991; Chen *et al.*, 1998). Recently, some information on the primary structure of mammalian 5-

lipoxygenase was derived from cDNA clones of mRNA that were isolated from various sources (Hoshiko *et al.*, 1990; Ohta *et al*, 1992). Nevertheless, information on the mechanistic details of the enzyme-catalyzed reaction (Pistirius and Axelrod, 1976; Glickman and Klinman, 1996) is scant due to the limitation in obtaining a sufficient quantity of the purified enzyme in a stable form from mammalian sources. It has been reported that the human 5-lipoxygenase activating protein could stimulate lipoxygenase from the potato tuber (Battu *et al.*, 1998), so for screening of the lipoxygenase inhibitor, potato lipoxygenase can be used instead of a human lipoxygenase. In order to screen 5-lipoxygenase inhibitors from microbial metabolites, we needed 5-lipoxygenase in a stable form. Therefore, the Korean red potato tubers were employed as the source of large amounts of 5-lipoxygenase in a stable form. This paper presents the results of the purification, characterization of the 5-lipoxygenase from the Korean red potato and the effect of the selected group specific reagents on the purified enzyme in order to investigate the essential amino acid residues of the enzyme.

Materials and Methods

Purification Procedure All purifications were performed at 4°C. One kilogram of a Korean red potato tuber was minced and homogenized in a Waring blender for 30 sec with 2 volumes of potassium phosphate buffer, pH 7.0, containing 2 mM sodium metabissulfite, 2 mM ascorbic acid, and 1 mM EDTA. The homogenate was filtered through two layers of cheesecloth and the filtrate was centrifuged at 10,000 g for 20 min. The pellet was discarded and the supernatant was subjected to $(NH_4)_2SO_4$ fractionation.

$(NH_4)_2SO_4$ fractionation Solid $(NH_4)_2SO_4$ was added under continuous stirring to the enzyme solution in order to give a final concentration of 15% saturation. After the entire amount of $(NH_4)_2SO_4$ was dissolved, the pH of the solution adjusted to 7.0 with 1 M NH_4OH, and the content stirred for 1 hr. The precipitated protein was removed by centrifugation at 15,000 g for 15 min and discarded. The resultant supernatant was brought to 45% saturation by the further addition of a solid $(NH_4)_2SO_4$, stirred for 1 hr, and then centrifuged at 15,000 g for 15 min. The pellet was resuspended in 40 mM potassium phosphate, pH 7.0 (Buffer A). This suspension was dialyzed for 24 hr against buffer A with three changes of buffer.

DEAE-Cellulose Column chromatography The dialyzed enzyme solution was centrifuged at 20,000 g for 30 min and the supernatant applied to DEAE-Cellulose column (4×20 cm) previously degassed and equilibrated with buffer A. Using the same buffer as an eluent, and a flow rate 25 ml/hr, the column was washed until the absorbance at 280 nm had dropped to below 0.2. No significant lipoxygenase activity was detected in the flow-through fractions. The eluting buffer was then changed to a linear KCl gradient of buffer A (400 ml each) containing 0.25 M KCl, and the effluent collected in 6 ml fractions. The fractions containing lipoxygenase activity were pooled and concentrated by ultrafiltration.

Assay of lipoxygenase The standard assay mixture (Spaeth *et al.*, 1992) contained 50 mM borate buffer, pH 9.0, and an enzyme in a total volume of 1 ml. The quartz cuvettes was maintained at 30°C. The reaction was initiated by an addition of 0.1 mM linoleic acid (10 μl of 10 mM stock solution in ethanol) and the increase in the absorbance at 234 nm (typical for the conjugated diene structure) was continuously monitored. To the reference cuvette was added 10 μl of ethanol before starting the reaction. Usually, the time course curve exhibited a sigmoidal shape (lag time before the maximal velocity). The sharpest slope in the middle of the reaction was taken as the initial velocity using 28,000 $M^{-1}cm^{-1}$ as a molar coefficient (Sun and McGuire, 1983). One unit was expressed as the activity of enzyme forming 1μmol of oxygenation product per min.

SDS-polyacrylamide gel electrophoresis and molecular weight determination The SDS-slab gel electrophoresis in a 10% gel was carried out according to Laemmli (1970). Protein samples in 10%(v/v) glycerol, 1%(w/v) SDS, 2%(v/v) β-mercaptoethanol and 0.0025% (w/v) bromophenol blue were heated in a boiling water bath for 5 min and then subjected to electrophoresis. Electrophoresis was carried out at 4°C and the voltage applied was 70 volts at stacking gel, 100 volts at running gel. The β-galactosidase, *E.coli* (116,000), phosphorylase b (97,400), bovine serum albumin (66,000), ovalbumin (45,000), carbonic anhydrase (29,000) were used as protein standards. Proteins were stained with Coomassie brilliant blue R-250 and destained with 5% methanol containing 7.5% acetic acid. For the determination of the native enzyme, gel filtration by Sepharose 6B column was carried out. Reference proteins were bovine throglobulin (669,000), horse spleen apoferritin (443,000), sweet potato β-amylase (200,000), yeast alcohol dehydrogenase (150,000), bovine serum albumin (66,000), and carbonic anhydrase (29,000).

Protein assay The concentrations of protein were measured by the method of Bradford (1976) and also estimated using the average protein absorbability of $A_{280\,(0.1\%)} = 0.15$.

Chemical modification of the purified lipoxygenase from red potato Several group specific chemical reagents at the concentration of 5-10 mM were used to investigate amino acids at the active site of lipoxygenase. Diethyl pyrocarbonate (Lee *et al.*, 1999b) N-acetylimidazole, phenylmethylsulfonyl fluoride, phenylglyoxal (Joo and Kim, 1998; Ahn *et al.*, 1999) N-bromosuccinimide (Lee *et al.*, 1999a), chloramine T, 2-hydroxy-5-nitrobenzyl bromide, ρ-hydroxymercurybenzoate, N-ethylmaleimide, Woodward's Reagent K and Iodine were preincubated with purified lipoxygenase at 30°C for 30 min and then the substrate was added under a standard assay condition, the residual enzyme activity was measured.

Protection of lipoxygenase against DEPC and WRK inactivation Purified lipoxygenase from a red potato was preincubated with linoleic acid for 1 min under standard assay conditions and then DEPC (5 mM) or WRK (10 mM) was added, then it was incubated for 20 min. The residual enzyme activity

was measured and compared with that of the chemical modification reaction mixture without preincubation with substrate.

Effect of pH and temperature The effects of pH on the activity and stability of the purified lipoxygenase from a red potato were determined. For stability, the enzyme solutions in 50 mM buffer at various pH values were incubated for 24 hr at 4°C. After adjustment of pH, the residual enzyme activities were assayed by the standard method. For activity, the enzyme was assayed by the standard method by changing the buffer in order to obtain the desired pH. The effects of temperature on the activity and stability of lipoxygenase from a red potato were also investigated. For stability, the enzyme solution in the borate buffer (50 mM, pH 9.0) was incubated for 30 min at various temperatures, and then the residual enzyme activities were assayed. For long storage the enzyme solution in potassium phosphate buffer containing 2 M $(NH_4)_2SO_4$ at -20°C was maintained and the residual activity was assayed. For activity, the enzyme was assayed at various temperatures by the standard assay method.

Results and discussion

Purification of 5-lipoxygenase The purification of 5-lipoxygenase from potato, hydroxyapatite chromatography and Mono Q column chromatography has been reported (Reddana, 1989), but these methods were not useful in our experiment.

Table 1. summarizes the results of the purification procedures of 5-lipoxygenase from the Korean red potato. The red potato homogenate was centrifuged and the supernatant was further precipitated with 15-45% saturated ammonium sulfate. The precipitate was absorbed by a DEAE-Cellulose column and eluted at 0.06-0.125 M KCl in buffer A (fraction number 20-29; Fig. 1). The enzyme was purified 35 fold to homogeneity with a specific activity of 1.05 U·mg of protein^{-1}. The purification procedure for lipoxygenase from a red potato was simple and resulted in high yields of protein. Stable and large quantities of purified lipoxygenase are required for the screening of the anti-inflammatory agent. So, the simple purification method is useful for the preparation of a pure lipoxygenase.

Molecular weight determination The subunit molecular

Table 1. Purification steps of 5-lipoxygenase from a red potato.

Purification	Total protein (mg)	Total activity (Unit)	Specific activity (U/mg)	Purification fold
Crude extract	23,000	690	0.03	1
Ammonium sulfate fractionation	3,200	576	0.18	6
DEAE-Cellulose chromatography	280	294	1.05	35

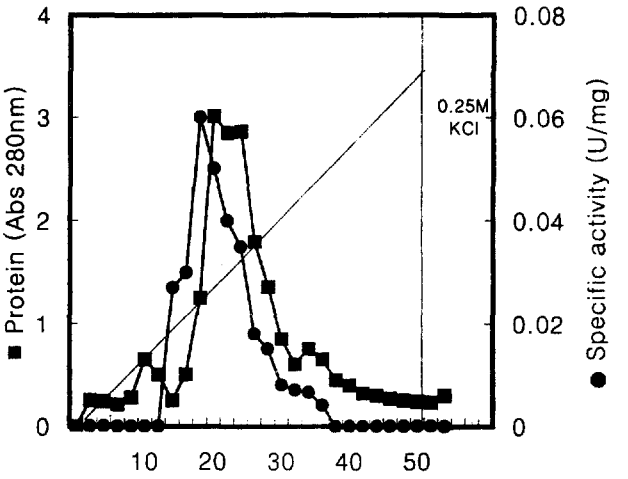

Fig. 1. Chromatogram of the lipoxygenase from a red potato on DEAE cellulose (4×20 cm). The column was eluted with 0 -0.25 M KCl containing potassium phosphate buffer, pH 7.0 at a flow rate 25 ml/hr; Fractions of 6.0 ml were collected.

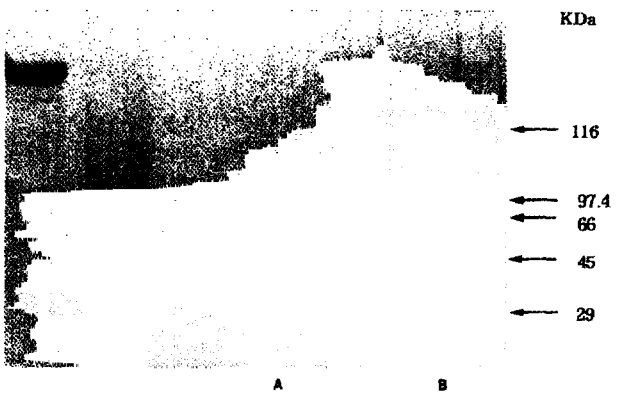

Fig. 2. SDS-polyacrylamide gel electrophoresis of lipoxygenase from a red potato. A; purified lipoxygenase from a red potato B; Molecular weight marker; phosphorylase b (97,400), bovine serum albumin (66,000), ovalbumin (45,000), carbonic anhydrase (29,000).

weight of purified lipoxygenase from a red potato was 38,000, which was determined in a SDS-polyacrylamide gel electrophoresis (Fig. 2). The L_2 form of 5-lipoxygenase from a potato was reported to have a Mr value of 35,000 (Reddanna *et al.*, 1989). The native molecular weight of the purified lipoxygenase by gel filtration of Sepharose 6-B was also 38,000 (Fig. 3). These results suggest that this enzyme is composed of only one polypeptide and its molecular weight is smaller than that of other lipoxygenases from human leukocyte and from rice leaf, which was reported to be 102,000. The microsomal lipoxygenase from a tomato was reported as 100,000 Dalton protein (Peever and Higgins, 1989).

Effect of pH and temperature For the effect of pH on enzyme activity the following buffers were used: 50 mM

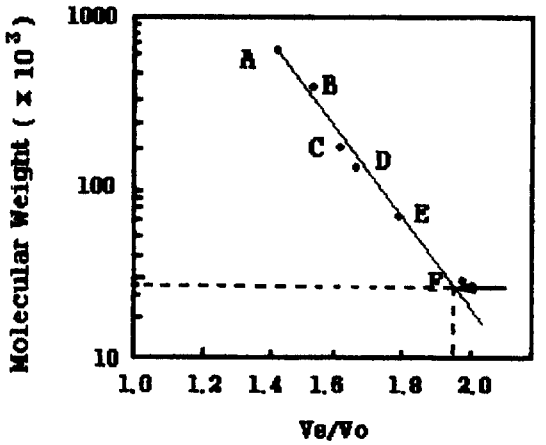

Fig. 3. Native molecular weight determination of lipoxygenase from a red potato by sepharose 6B gel filtration. Protein standards were A; bovine throglobulin (669,000), B; horse spleen apoferritin (443,000), C; sweet potato β-amylase (200,000), D; yeast alcohol dehydrogenase (150,000), E; bovine serum albumin (66,000), F; carbonic anhydrase (29,000). The arrow shows molecular weight of lipoxygenase purified from a red potato.

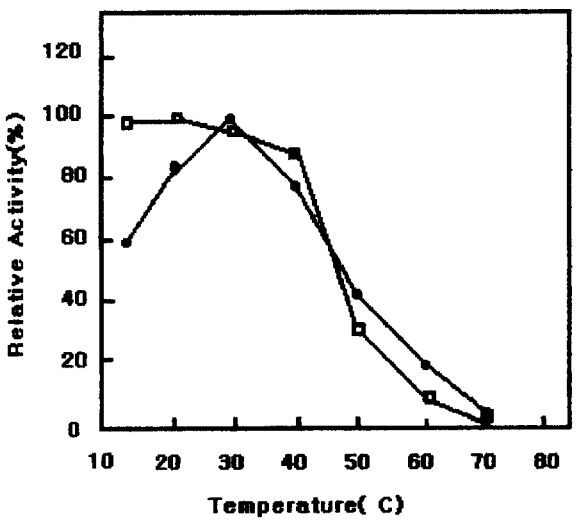

Fig. 4. Effect of pH on the activity (●) and stability (❑) of purified lipoxygenase from a red potato. For stability, the enzyme solutions in 50 mM buffer at various pH values were incubated for 24 hr at 4°C. After adjustment of pH, the residual enzyme activities were assayed by the standard method. For activity, the enzyme was assayed by the standard method by changing the buffer in order to obtain the desired pH.

sodium acetate buffer for pH 4-6, 50 mM potassium phosphate buffer for pH 6-8, 50 mM Tris-HCl buffer for pH 8-9, 50 mM sodium borate buffer for 9-11. The optimal pH for an enzyme reaction was found to be 9.0. The enzyme was more active in an alkaline condition than in an acidic one. The enzyme was stable at pH 7.0-10.0 at 4°C (Fig. 4). The enzyme was most active at 30°C. It was quite stable at 40°C for 30 min and retained over 90% of the original activity under these

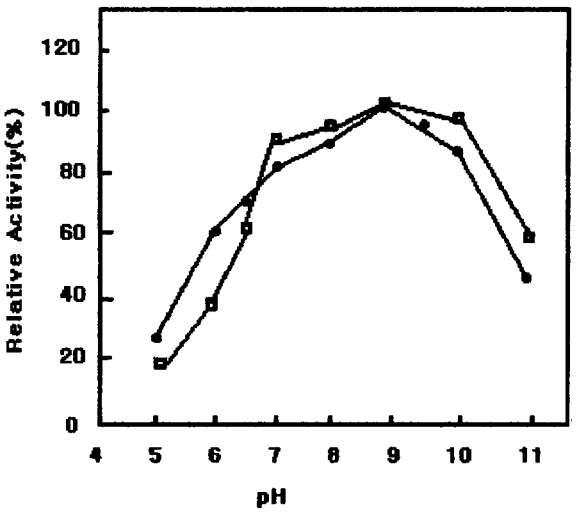

Fig. 5. Effect of temperature on the activity (●) and stability (❑) of lipoxygenase from a red potato. For stability, the enzyme solution in borate buffer (50 mM, pH 9.0) was incubated for 30 min at various temperatures, then the residual enzyme activities were assayed. For activity, the enzyme was assayed at various temperatures by the standard assay method.

conditions. The enzyme, however, lost its activity completely after 30 min of incubation at 70°C (Fig. 5). The purified enzyme with 2 M $(NH_4)_2SO_4$ in potassium phosphate buffer, pH 7.0, was very stable for 5 months at -20°C. Because the purified lipoxygenase was very stable it could be useful for the screening of a lipoxygenase inhibitor (Galpin *et al.*, 1976; Hook *et al.*, 1990; Kitamura *et al.*, 1986). The purified lipoxygenase from a red potato appeared to differ in its optimal pH to that of lipoxygenase from tomato and rice. The optimal pH for the microsomal and soluble form of lipoxygenase from a tomato is known to be 4.5-8.0 and 6.0, respectively (Todd *et al*, 1986a). The lipoxygenase isoenzymes of rice embryo are reported to have optimal pH of 4.5, 5.5 and 7.0 (Ohta *et al.*, 1992) The soybean lipoxygenase-1 (Clapp *et al.*, 1985) shows an optimal pH of 9.0, the same as that of the purified enzyme from a red potato.

Substrate specificity and kinetic analysis The substrate specificity of the purified enzyme was examined by the standard enzyme assay, except that linoleic acid was substituted with various substrates to a final concentration of 0.1 mM. Only the linoleic and arachidonic acids acted as a substrate of purified lipoxygenase from the Korean red potato (Table 2.). The rate of substrate conversion was approximately eight times greater with linoleic acid than with arachidonic acid. The following compounds were not utilized as substrates of lipoxygenase from a red potato at a detectable rate: phosphatidylcholine, phosphatidic acid, diacylglycerol and oleic acid. Values for the apparent Km and Vmax of the purified lipoxygenase, calculated from the Lineweaver-Burk double reciprocal plot (Fig. 6.) with linoleic acid as substrates,

Table 2. Substrate preference of lipoxygenase from a red potato.

Substrate	Activity (mol/min, mg protein)
Linoleic acid	1.05
Arachidonic acid	0.13
Phosphatidylcholine	ND[a]
Phosphatidic acid	ND
Diacylglycerol	ND
Oleic acid	ND

[a]Not detectable

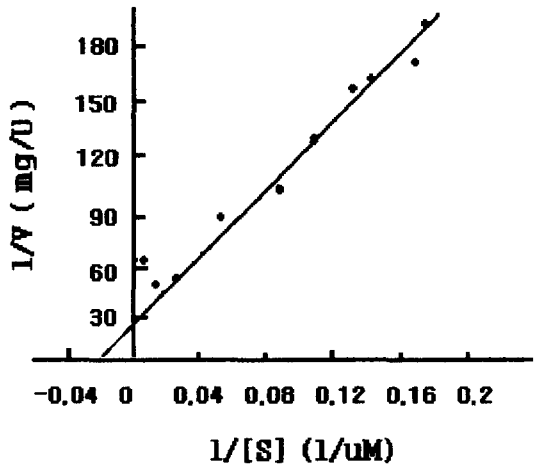

Fig. 6. Lineweaver-Burk plot of the lipoxygenase from a red potato.

are 48 μM and 0.03 μM per minute per milligram of protein, respectively. The purified lipoxygenase from a red potato had a higher affinity to linoleic acid than that of rice seedlings and tomato microsome. The Km values of lipoxygenases from rice seedlings (Ohta *et al*, 1986) and from tomato microsome (Todd *et al.*, 1990) were reported as 59 μM and 0.52 mM, respectively.

Effect of divalent cations and chelating agents The known metal chelating agents that were tested (2 mM KCN, 1 and 10 mM EDTA, and 1 mM NaN_3) showed no significant effect on the lipoxygenase activity under the assay condition. This suggests that no easily removed divalent metal is necessary for enzyme activity. Several divalent cations were tested for their effect on the purified lipoxygenase activity under the assay conditions (Table 3.). The lipoxygenase from human leukocyte is known to require Ca^{++} for maximal enzyme activity (Samuelsson *et al.*, 1987); whereas, the purified enzyme from a red potato was unaffected by Ca^{++}, Fe^{++}, Fe^{+++} and Zn^{++}. However, Co^{++}, Cu^{++} and Ni^{++} reduced the enzyme activity by 55%, 34% and 40%, respectively.

Chemical modification of the purified lipoxygenase from red potato Several group specific chemical reagents were used to investigate the amino acids at the active site of lipoxygenase. The results of these studies are summarized in Table 4. The lipoxygenase activity from a red potato was unaffected by p-hydroxymecuribenzoate and N-ethylmaleimide. This suggests that the sulfhydryl groups are not involved in the catalytic center of the enzyme. Treatment of the enzyme with group-specific reagents DEPC, WRK resulted in a residual activity of less than 15%. This shows histidine, aspartic acid or glutamic acid residues are at or near the active site of the enzyme. Time and concentration dependent inactivation by the histidine specific DEPC was demonstrated. Semilogarithmic plots of residual activity as a function of time were linear, indicating that the inactivation by

Table 3. Effect of chelating agents and metal ions on the activity of lipoxygenase from a red potato.

Metal ion* or chelating agents	Relative activity (%)
None	100
KCN(2mM)	95
EDTA(1 and 10mM)	98
NaN_3(1mM)	98
Co^{++}	55
Mn^{++}	87
Ni^{++}	40
Zn^{++}	90
Mg^{++}	72
Fe^{++}	98
Cu^{++}	34
Fe^{+++}	115
Ca^{++}	98

*Metal ions were used as chloride salts and at the concentration of 1 mM or 10 mM.

Table 4. Effects of chemical modifiers on the lipoxygenase from a red potato.

Chemicals	Target amino acid	Inhibitory concentration (mM)	Residual activity (%)
None			100
Dimethyl pyrocarbonate	His	10	15
N-acetylimidazole	Tyr	10	97
Phenylmethylsulfonyl fluoride	Ser	5	87
Phenylglyoxal	Arg	5	78
N-Bromosuccinimide	Trp, Cys, Tyr	5	92
Chloramine T	Met	5	100
2-Hydroxy-5-nitrobenzyl bromide	Trp	5	100
p-Hydroxymercurybenzoate	Cys	5	100
N-ethylmaleimide	Cys	10	100
Woodward's Reagent K	Asp, Glu	10	12
Iodine	Tyr	10	90

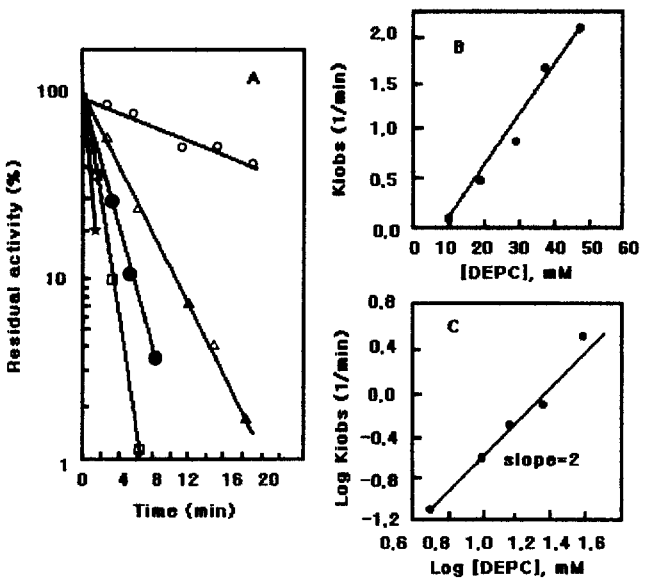

Fig. 7. The inactivation of the lipoxygenase of a red potato with DEPC. A. Purified enzyme in 0.1 M borate buffer, pH 9.0 was incubated with 10 (○), 20 (△), 30 (●), 40 (□) mM, and 50 (★) mM of the reagent. Samples were removed as a function of time and residual enzyme activity was determined. B. Determination of the rate constant of inactivation. C: Apparent order of reaction with respect to reagent concentration.

Table 5. Protection of the lipoxygenases from a red potato by linoleic acid from chemical modification.

Incubation time (min)	Residual activity (%)			
	WRK (10mM)		DEPC (5mM)	
	A*	B*	A	B
0	100	100	100	100
5	36	68	35	75
10	25	54	26	62
15	18	44	22	57
20	12	42	15	50

#A; without preincubation with linoleic acid.
B; with preincubation with linoleic acid.

DEPC is first order process (Fig. 7A). The slope could be used for calculation of apparent pseudo-first-order constants. A plot of these constants against the DEPC concentration was linear (Fig. 7B). Analysis of the order of inactivation with respect to the DEPC concentration, by the method of Levy *et al.*(1963), yielded a slope of 2.0 (Fig. 7C). This indicates that two molecules of DEPC bind to one molecule of enzyme when inactivation occurs. The effect of WRK on enzyme was investigated in the same way. It seems likely that two histidine residues, and two Glu/Asp carboxyl groups, play an important role in the catalytic site of the enzyme.

Protection of lipoxygenase against DEPC and WRK inactivation The effect of the substrate on the DEPC and WRK inactivation of the enzyme was investigated. When the enzyme was incubated with a linoleic acid prior to the DEPC or WRK treatment, the enzyme was protected against inactivation (Table 5.). The protection of inactivation by linoleic acid suggested that the functional group of the enzyme, modified by DEPC or WRK, must be in the area of the linoleic acid binding site and hence at, or near, the active site of the enzyme.

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References

Ahn, J. Y., Cho, E. H., Lee, K. S., Choi, S. Y. and Cho S. W. (1999) Chemical modification of Brain Glutamate Dehydrogenase isoprotein with Phenyglyoxal. *J. Biochem. Mol. Biol.* **32**, 515-520.

Battu, S., Cook-Moreau, J. and Beneytout, J. L. (1994) Stabilization of potato tuber on talc. *Biochem. Biophys. Acta.* **1211**, 270-276.

Battu, S., Moalic, S., Rigaud, M. and Beneytout, J. L. (1998) Linoleic acid peroxidation by Solanum tuberosum lipoxygenase was activated in the presence of human 5-lipoxygenase -activating protein. *Biochim. Biophys. Acta.* **1392**, 340-350.

Butovich, I. A., Lukyanova, S. M. and Reddy, C. C. (1998) Oxidation of linoleyl alcohol by potato tuber lipoxygenase: possible mechanism and the role of carboxylic group in substrate binding. *Biochem. Biophys. Res. Commun.* **249**, 344-349.

Chen, X., Reddanna, P. Reddy, G. R. Kidd, R., Hildenbrandt, G. and Reddy, C. C. (1998) Expression, purification, and characterization of a recombinant 5-lipoxygenase from potato tuber. *Biochem. Biophys. Res. Commun.* **243**, 438-443.

Clapp, C. H., Banerjee, A. and Rotenberg, S. A. (1985) Inhibition of soybean lipoxygenase 1 by N-Alkylhydroxyamines. *Biochemistry.* **24**, 1826-1830.

Evans, A. T., Formukong, E. A. and Evans, F. J. (1987) Actions of cannabis constituents on enzymes of arachidonate metabolism. *Antiinflammatory Potential.* **36**, 2035-2039.

Galpin, J. R., Tielens, G. M., Veldink, G. A. and Boldingh, J. (1976) On the interaction of some catechol derivatives with the iron atom of soybean lipoxygenase. *FEBS LETTERS.* **68**, 179-185.

Gardner, H. W. (1991) Recent investigation into the lipoxygenase pathway of plants. *Biochim. Biophys Acta.* **1084**, 221-225.

Glickman, M. H. and Klinman. J. P. (1996) Lipoxygenase reaction mechanism Demonstration that hydrogen abstraction from substrate precedes dioxygen binding during catalytic turnover. *Biochemistry* **35**, 12882-12886.

Hoshiko, S., Radmark, O and Samuelsson, B. (1990) Characterization of the human 5-lipoxygenase gene promoter.

Proc. Natl. Acad. Sci. USA **87,** 9073-9077.

Hook, D., Yacobucci, J. J., Lee, M. and Hesler, G (1990) Identification of the inhibitory activity of carbazomycins B and C against 5-lipoxygenase, A new activity for these compounds. *J. Antibiot.* **43**, 1347-1352.

Huaaey, H. J. and Yisdale, M. J. (1996) Inhibition of tumor growth by lipoxygenase inhibitors. *Brit. J. Cancer.* **74**, 683-688.

Joo, H. S. and Kim, S. S. (1998) Chemical modification of Serratia marcescences Catabolic-Acetolactate Sythase. *J. Biochem. Mol. Biol.* **31**, 139-143.

Kemal, C., Louis, F. P., Krupinski, O. R. and Shorter, A. L. (1987) Reductive Inactivation of soybean lipoxygenase 1 by catechols: A possible mechanism for regulation of lipoxygenase activity. *Biochemistry.* **26**, 7064-7068.

Kitamura, S., Hashizume, K., Lida, T., Miyashira, E., Shirahata, K. and Kase, E. (1986a) Studies on lipoxygenase inhibitors. *J. Antibiot.* **39**, 1160-1165.

Kitamura, S., Lida, T., Shirahata, K. and Kase, H. (1986b) Studies on lipoxygenase inhibitors I. MY3-469, a potent and selective inhibitor of 12-lipoxygenase, produced by *Streptoverticillum hadanonense* KY11449. *J. Antibiot.* **39**, 589-595.

Laemmli, U. K. (1970) Cleavage of structural protein during the assembly of the head of bacteriophage T4. *Nature* **227**, 680-685.

Lee, B. R., Jeon, S. G., Bahn, J. H., Choi, K. S. Yoon, B. H., Ahn, Y. K., Choi, E. A., Lee, K. S., Cho, S. W. and Choi, S. Y. (1999a) Chemical modification of Bovine Brain Succinic Semialdehyde Reductase by Diethylpyrocarbonate. *J. Biochem. Mol. Biol.* **32**, 254- 258.

Lee, B. R., Bahn, J. H, Jeon. S. G., Ahn, Y. K., Yoon, B. H., Kwon, H. Y., Kwon, O. S. and Choi, S. Y. (1999b) Chemical modification of Porcine Brain myo-Inositol Monophosphate Phosphatase by N-bromosuccinimide. *J. Biochem. Mol. Biol.* **32**, 294-298.

Mylari, B. L., Carty, T. J., Moore, P. F. and Zembrowski, W. J. (1990) 1,2-Dihydro-1-oxopyr-rolo[3,2,1-kl]phenothiazine-2-carboxamides and Congeners, dual cyclooxygenase/5-lipoxygenase inhibitors with anti-inflammatory activity. *J. Med. Chem.* **33**, 2019-2023.

Nakatsuka, M. and Osawa, Y. (1994) Selective inhibition of the 12-lipoxygenase pathway of arachidonic acid metabolism by L-arginine or sodium nitroprusside in the intact human platelets. *Biochem. Biophys. Res. Commun.* **200**, 1630-1634.

Neichi, T., Koshihara, Y. and Murota, S. (1983) Inhibitory effect of esculetin on 5-lipoxygenase and leukotriene biosynthesis. *Biochim. Biophys. Acta.* **753,** 130-135.

Nihel, Y., Yamamoto, H., Hasegawa, M., Hanada, M., Fukagawa, Y. and Oki, T. (1993) Epocarbazolines A and B, Novel 5-lipoxygenase inhibitors, Taxonomy, fermentation, isolation, structures and biological activities. *J. Antibiot.* **46**, 25-29.

Ohta, H., Ida, S., Mikami, B. and Morita, Y. (1986) Changes in lipoxygenase of rice seedling during germination. *Plant Cell Physiol.* **27**, 911-918.

Ohta, H., Shirano, Y., Tanaka, K., Morita, Y. and Shibata, D. (1992) cDNA cloning of rice lipoxygenase L-2 and characterization using an active enzyme expressed from the DNA in *Escherichia coli. Eur. J. Biochem. FEBS.* **206**, 331-336.

Percival, M. D. (1991) Human 5-lipoxygenase contains an essential iron. *J. Biol. Chem.* **266**, 10058-10063.

Piomelli, A. A., Dale, V. N., Siegelbaum, S. A., Kandel, E. R., Schwartz, J. H. and Belardetti, F. (1987) Lipoxygenase metabolites of arachidonic acid as second messengers for presynaptic inhibition of *Aplisia* sensory cells. *Nature.* **328**, 38-44.

Pistorius, E. K. and Axelrod, B. (1976) Evidence for participation of iron in lipoxygenase reaction from optical and electron spin resonance studies. *J. Biol. Chem.* **251,** 7144-7149.

Reddana, P., Whelan, J., Maddipati, K. R. and Reddy, C. C. (1989) Purification of Arachidoante 5-Lipocygenase from Potato Tubers. *Method. Enzymol.* **187**, 268-277.

Rouzer, C. A., Matsumoto, T. and Samuelsson, B. (1985) Single protein from human leukocytes possesses 5-lipoxygenase and leukotriene A_4 synthetase activities. *Proc. Natl. Acad. Sci. USA* **83**, 857-862.

Samuelsson, B. (1983) Leukotriens; a new class of mediators of immediate hypersensitivity reactions and inflammation: In Advances in Prostaglandin, Thromboxane, and Leukotriene Research, pp. 1-6, Raven Press, New York.

Schneider, C., Schreier, P. and Herderich, M. (1997) Analysis of lipoxygenase-derived fatty acid hydroperoxides by electrospray ionization tandem mass spectrometry. *Lipid.* **32**, 331-335.

Shimizu, T., Izumi, T., Seyame, Y., Tadokoro, K., Rasmark, O. and Samuelsson, B. (1986) Characterization of leukotriene A_4 synthase from murine mast cells: evidence for its identity to arachidonate 5-lipoxygenase. *Proc. Natl. Acad. Sci. USA.* **83**, 4175-4179.

Skeiya, K. and Okuda, H. (1982) Selective inhibition of platelet lipoxygenase by esculetin. *Biochem. Biophys. Acta.* **713,** 68-73.

Spaethe, S., Snyder, D. W., Pechous, P. A., Clarke, T. and Vanalstyne, E. L. (1992) Guinea pig whole blood 5-lipoxygenase assay: utility in the assessment of potential 5-lipoxygenase inhibitors. *Biochem. Pharmacol.* **43**, 377-382.

Sun, F. F. and McGuire, J. C. (1983) Inhibition of human neutrophil arachidonate 5-lipoxygenase by U-60,257. *Prostaglandins.* **26**, 211-216.

Todd, J. F., Paliyath, H. and Thompson, J. E. (1990) Characteristics of a membrane-associated lipoxygenase in tomato fruit. *Plant. Physiol.* **94**, 1225-1232.

Walker, J. R., Boot, J. R., Cox, B. and Dawson, W. (1980) Inhibition of the release of slow reacting substance of anaphylaxis by inhibitors of lipoxygenase activity. *J. Pharm. Pharmacol.* **32**, 866-870.

Yamamoto, S., Suzuki, H. and Ueda, N. (1997) Arachidonate 12-lipoxygenases. *Prog. Lipid Res.* **36**, 23-29.

Yoshimoto, T., Furukawa, M., Yamamoto, S., Horie, T. and Kohno, S. (1983) Flavonoids: potent inhibitors of arachidonate 5-lipoxygenase. *Biochem. Biophys. Res. Commun.* **116**, 612-618.

Zhang, L. Y. and Hamberg, H (1996) Specificity of two lipoxygenase from rice: Unusual regiospecificity of a lipoxygenase isoenzyme. *Lipids.* **31**, 803-808.