Inactivation of Tea Leaf Hydroperoxide Lyase by Fatty Acid Hydroperoxide

Kenji Matsui,* Tadahiko Kajiwara, and Akikazu Hatanaka

Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Yamaguchi 753, Japan

Tea leaf hydroperoxide lyase (HPO lyase) was rapidly and irreversibly inactivated by linoleic acid 13-hydroperoxide [13(*S*)-hydroperoxy-9(*Z*),11(*E*)-octadecadienoic acid (LA 13-HPO)]. The rate of the inactivation was directly dependent upon the concentration of hydroperoxide, and its second-order rate constant at pH 5.5 and 25 °C was 265 M^{-1} s^{-1}. Linoleic acid 9-hydroperoxide [9(*R*)-hydroperoxy-10(*E*), 12(*Z*)-octadecadienoic acid], which is little catalyzed by tea leaf HPO lyase, also inactivated the enzyme, but the other organic hydroperoxides such as cumene, *tert*-butyl hydroperoxide, and H_2O_2 did not. Inactivation by LA 13-HPO was protected either by dithiothreitol or by LA 13-hydroxide, which suggests that the inactivation is caused by destruction of an essential SH group near the reaction center of the enzyme. Hydrophobic radical scavengers such as α-tocopherol and nordihydroguaiaretic acid effectively protected HPO lyase from the inactivation, but mannitol and diethylenetriaminepentaacetic acid showed no effect.

INTRODUCTION

Hydroperoxide lyase (HPO lyase) is the enzyme that cleaves fatty acid hydroperoxide to form short-chain aldehydes and is ubiquitous in the plant kingdom. The hydroperoxides (HPOs) of either linoleic or linolenic acids are the usual substrates of the enzyme. This enzyme has considerable impact on the flavor of certain leaves, fruits, and vegetables. Flavors described as grassy, beany, and leafy have been attributed to the products, and in some cases they cause pleasant and in other cases unpleasant flavors (Gardner, 1985). Although the importance of this enzyme in food processing is well recognized, investigation on the properties and reaction mechanisms of the enzyme have been scarcely reported. In tea leaves, 13(*S*)-hydroperoxy-9(*Z*),11(*E*)-octadecadienoic acid (LA 13-HPO) was formed from linoleic acid by lipoxygenase, and this HPO was cleaved into *n*-hexanal and 12-oxo-9(*Z*)-dodecenoic acid by HPO lyase. *n*-Hexanal and 3(*E*)-hexenal (formed from linolenic acid) were known to be important components in tea flavor. Recently, we purified HPO lyase from tea leaves and elucidated some properties (Matsui et al., 1991). Tea leaf HPO lyase preferentially cleaves fatty acid 13-HPO, while the 9-isomer is little catalyzed. Because Hg^{2+} and *p*-(chloromercuri)benzoic acid strongly inhibited the activity, participation of an SH group in the reaction was proposed. During further investigation to reveal the reaction mechanism, we found that the enzyme was rapidly inactivated by its natural substrates, fatty acid HPOs. HPOs are known to be potentially reactive to cause deterioration of food proteins or amino acids (Gardner, 1979), but the effect of HPOs on enzyme activity has been little reported. Inactivation of HPO lyase may be needed in the processing of some edible plants. In this paper the kinetics and mechanism of inactivation of tea leaf HPO lyase by fatty acid HPOs are examined.

MATERIALS AND METHODS

Materials. Linoleic acid (LA) 13-hydroperoxide (HPO) and LA 9-HPO were prepared from LA (Wako Pure Chemicals, Osaka, Japan) by using soybean lipoxygenase (Sigma, type I) and potato tuber lipoxygenase, respectively, as described (Matsui et al., 1989). Both the HPOs contained less than 10% of geometrical and positional isomers. LA 13-hydroxide was prepared by reduction of LA 13-HPO by sodium borohydride. The other chemicals were of reagent grade.

Methods. *Enzyme Preparation.* HPO lyase was purified from tea leaves grown in the University's tea garden essentially as previously described (Matsui et al., 1991), but the first DEAE-Cellulofine column was omitted. The specific activity of HPO lyase was 18 units/mg under the standard assay condition described below. Although HPO lyase activity was separated by hydroxylapatite chromatography into HPO lyases I and II, we used only HPO lyase I because this isoform was thought to be an intact form; in this paper HPO lyase I is simply denoted HPO lyase. Alcohol dehydrogenase (120 units/mg, from yeast) was purchased from Oriental Yeast Co. (Osaka, Japan).

Determination of Enzyme Activities. HPO lyase activity was measured by the loss of absorption at 236 nm caused by conjugated diene of LA 13-HPO at 25 °C. A typical reaction mixture contained 50 mM 2-(*N*-morpholino)ethanesulfonic acid (MES)–KOH (pH 5.5), 40 μM LA 13-HPO, and enzyme in a total volume of 1 mL. Alcohol dehydrogenase was measured by the loss of absorption at 340 nm caused by NADH at 25 °C (Pietruszko et al., 1973). A typical reaction mixture contained 100 mM phosphate buffer (pH 7.0), 10 mM β-mercaptoethanol, 100 mM acetaldehyde, 0.1 mM NADH, and enzyme in a total volume of 1 mL. One unit of the activities was expressed as the amount of enzyme consuming 1 μmol of LA 13-HPO or NADH within 1 min by using 25 000 or 6220 M^{-1} cm^{-1} as the extinction coefficient, respectively. Inactivation kinetics of HPO lyase by LA 13-HPO was followed by determination of instantaneous reaction velocities at 15- or 20-s intervals by a Shimadzu (Kyoto, Japan) MPS-2000 multipurpose spectrophotometer at 25 °C. Otherwise, HPO lyase or alcohol dehydrogenase was incubated with HPO for 10 min, and then the residual activity of the mixture was measured after 10- or 100-fold dilution with solution containing respective substrate. Details are described in the figures and tables.

Protein and HPO Determinations. The amount of HPO group was determined according to the ferrous thiocyanate method as described (Wurzenburger and Grosch, 1984). Protein content was determined according to a modification of Lowry's method (Dulley and Grieve, 1975) using bovine serum albumin as standard.

RESULTS AND DISCUSSION

For determination of HPO lyase activity, we previously quantified the amount of a volatile product, *n*-hexanal, formed from LA 13-HPO in the head space of a reaction vessel by GLC. However, in this study the kinetics of the HPO lyase reaction were followed by the loss of absorption at 236 nm caused by LA 13-HPO instead of by a head space analysis method. As shown by the traces in Figure

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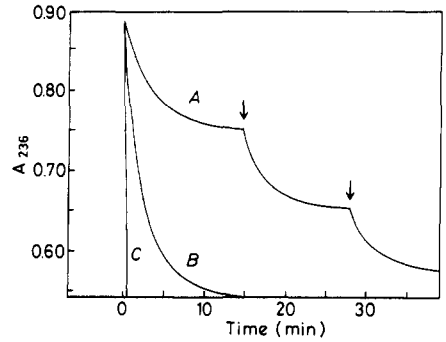

Figure 1. Breakdown of LA 13-HPO by tea leaf HPO lyase. The reaction mixture contained 35.6 μM LA 13-HPO and 1.74 (trace A), 4.64 (trace B), or 17.4 (trace C) nM HPO lyase in 50 mM MES–KOH (pH 5.5) (total volume, 1 mL). Reactions were initiated by the addition of HPO lyase. Where arrow indicates, additional 1.74 nM HPO lyase was added to the reaction mixture.

1, the addition of the enzyme to LA 13-HPO resulted in loss of absorption with no apparent lag phase. No other change in absorbance between 190 and 350 nm was observed. Reaction conditions were optimized to obtain initial slopes of traces which were well resolved enough to determine an initial reaction velocity. On the basis of this assay procedure, it was demonstrated that HPO lyase activity obeys normal Michaelis–Menten kinetics and the K_m of LA 13-HPO for HPO lyase was 1.46×10^{-5} M at the optimum pH of 5.5. When a low concentration of the enzyme was used, the reaction velocity was gradually decreased and finally reduced to nil within 15 min prior to all of the available substrate being used (traces A and B in Figure 1). Increasing the LA 13-HPO concentration did not cause further reaction; however, a secondary addition of the same amount of fresh enzyme did induce renewed reaction (trace A). The extents of both the primary and secondary initial rates of reaction were almost the same. Inactivation of HPO lyase was also evident when loss of the HPO group was monitored by using the ferrous thiocyanate method. Extensive dialysis of the inactivated enzyme against 50 mM MES–KOH (pH 5.5) did not restore the activity. Dialysis against the same buffer containing 1 mM dithiothreitol also failed to restore the activity. These results indicated that inactivation of HPO lyase by LA 13-HPO was irreversible. It has been reported that tea leaf HPO lyase utilizes only 13-HPOs of linoleic and linolenic acid but not the respective 9-HPOs (Hatanaka et al., 1982). Furthermore, this enzyme shows enantioselectivity and only catalyzes 13(*S*)-HPO isomers (Kajiwara et al., 1982). Although the LA 13-HPO used in this study contained a small amount of enatiomeric, geometrical, and positional isomers which would remain in the mixture and probably inhibit the reaction in a competitive manner, the commencement of renewed reaction with the addition of the same amount of fresh enzyme indicated that the inactivation of HPO lyase was not responsible for increasing the compositions of non-catalyzable HPO isomers. The activity did not decrease even after a 30-min incubation under the same condition without HPO, and furthermore, incubation of HPO lyase with either or both of the products, *n*-hexanal and 12-oxo-9(*Z*)-dodecenoic acid, did not affect the activity. These results indicated that HPO lyase was progressively and irreversibly inactivated by its substrate, LA 13-HPO.

To obtain inactivation kinetics, instantaneous velocities were determined at 15- or 20-s intervals from tracings of reaction progress curves. Because lowering the substrate concentration during catalysis would greatly affect the reaction velocity of HPO lyase and thus inactivation kinetics, a low concentration of the enzyme and a high

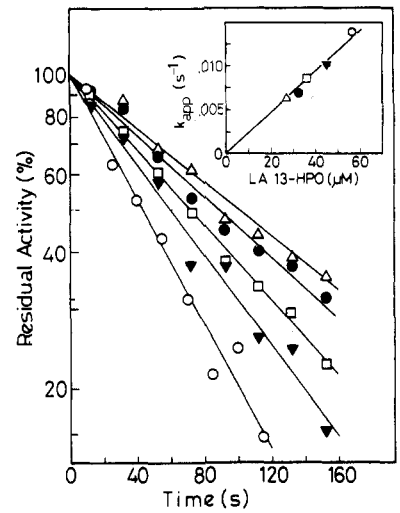

Figure 2. Inactivation of tea leaf HPO lyase as a function of the concentration of LA 13-HPO. Tea leaf HPO lyase, at 1.16 nM in 50 mM MES–KOH (pH 5.5) and 25 °C was exposed to LA 13-HPO in a total volume of 1 mL. Inactivation was followed by determination of instantaneous reaction velocities at 15- or 20-s intervals. The initial concentrations of LA 13-HPO were 26.8 (△), 32.3 (●), 36.8 (□), 45.1 (▼), and 56.9 (○) μM. Plotted on the ordinate is the logarithm of the concentration of active enzyme remaining at the indicated times after addition of LA 13-HPO. (Inset) Apparent rate constant of inactivation (k_{app}) plotted against the initial concentration of LA 13-HPO.

concentration of the substrate (>2 K_m) were used. Inactivation of HPO lyase proceeded in a time- and concentration-dependent manner. Semilogarithmic plots of the residual activities were linear and obeyed the first-order rate law (Figure 2). Plotting the apparent first-order rate constant (k_{app}) vs initial concentration of LA 13-HPO gave a reasonably good straight line. This indicated that the kinetics of inactivation were consistent with the simple model shown in (Fitzpatrick and Villafranca, 1986)

$$E + I \overset{K}{\rightarrow} E_{inact} \quad (1)$$

The second-order rate constant for inactivation was determined to be 265 M^{-1} s^{-1}. Little and O'Brien (1968) reported that the values of the second-order rate constants for the oxidation of thiols in bovine serum albumin were 140 and 500 M^{-1} s^{-1} (for native and denatured albumins, respectively). These values were thought to be comparable with those of inactivation of HPO lyase because destruction of an SH group of HPO lyase was thought to be responsible for the inactivation (see below). However, in the reaction mixture they used, cytochrome *c* was involved as a catalyst. Heme compounds were known to convert HPO to a highly reactive form such as radical species (Gardner, 1975; Chamulitrat et al., 1989). Because fatty acid HPO per se is not so reactive as to destroy an amino acid residue in a protein, it was thought to be essential to convert LA 13-HPO to a highly reactive form in inactivating HPO lyase. The high second-order rate constant obtained with HPO lyase and LA 13-HPO suggested that HPO lyase by itself behaves as a catalyst to activate LA 13-HPO, although we have not obtained any direct evidence.

As shown in Figure 3, HPO lyase showed a symmetrical pH–activity profile having a pH optimum at 5.5, while a higher degree of inactivation was observed in more acidic media and showed no optimum. Experiment below pH 4 was not carried out because the insolubility of LA 13-HPO interfered with an accurate estimation. The marked difference between pH dependencies of the two parameters suggested that a mechanism to inactivate HPO lyase,

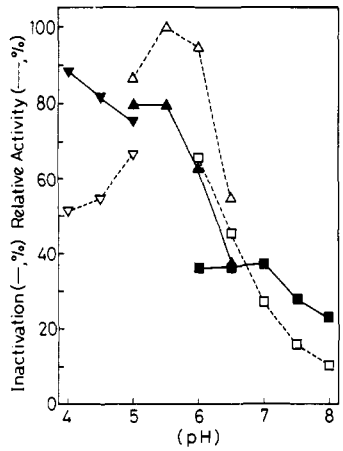

Figure 3. Degree of inactivation of HPO lyase by LA 13-HPO (straight line) and relative activity of HPO lyase (dotted line) as a function of pH. Tea leaf HPO lyase (46.4 nM) was incubated at 25 °C for 10 min with 40 μM LA 13-HPO at the indicated pH. Residual activity was determined by 10-fold dilution of the mixture with 33 μM LA 13-HPO in 50 mM MES-KOH (pH 5.5). Control experiment without LA 13-HPO was also carried out at each pH, and percent of inactivation of respective control activity was plotted. Relative activity of HPO lyase was determined with 30 μM LA 13-HPO. Buffers used were 20 mM sodium acetate (pH 4.0–5.0; ▼, ▽), 20 mM MES-KOH (pH 5.0–6.5; ▲, △), and 20 mM sodium phosphate (pH 6.0–8.0; ■, □).

Table I. Effect of HPOs and Related Compounds on the Activity of HPO Lyase and Alcohol Dehydrogenase[a]

	residual act., %	
HPO added	HPO lyase	alcohol dehydrogenase
none	100 ± 7.5	100 ± 16.5
linoleic acid 13-hydroperoxide	24.5 ± 1.0	10.9 ± 3.7
linoleic acid 9-hydroperoxide	15.0 ± 2.0	nd[b]
linoleic acid 13-hydroxide	90.7 ± 2.4	nd
tert-butyl hydroperoxide	98.2 ± 8.3	9.4 ± 3.0
(96 μM)	106.9	nd
cumene hydroperoxide	103.3 ± 4.9	8.3 ± 0.8
(96 μM)	98.6	nd
hydrogen peroxide	104.6 ± 3.7	60.7 ± 15.0

[a] HPO lyase (46.6 nM) or yeast alcohol dehydrogenase (46.5 nM) was incubated with 40 μM (or 96 μM) of HPOs or hydroxide in 50 mM MES-KOH (pH 5.5) for 10 min at 25 °C. Residual activity was determined after 10-fold dilution of the mixture with 50 mM MES-KOH (pH 5.5) containing 29.5 μM LA 13-HPO or 100-fold dilution with 100 mM phosphate buffer (pH 7.0) containing 0.1 M acetaldehyde, 0.1 mM NADH, and 10 mM 2-mercaptoethanol, for HPO lyase or alcohol dehydrogenase, respectively. Values are means ± SD of three repeats. [b] nd, not determined.

probably involving activation of fatty acid HPO, was not completely the same as a normal reaction mechanism that usually forms the products from fatty acid HPO. The pH–inactivation curve revealed one pK value of about 6.5. Because any functional group in LA 13-HPO has such a pK value, this indicates that a functional group in the enzyme having a pK at around 6.5 participates in the inactivation. Matsushita et al. (1970) reported almost the same pH-dependent profile with inactivation of pancreas ribonuclease by autoxidized LA, although the inactivation mechanism of the ribonuclease has not been specified.

Inactivation of HPO lyase was investigated with the other HPOs and related compounds (Table I). To estimate the sensitivity and specificity of HPO lyase against various HPOs, inactivation of yeast alcohol dehydrogenase caused by these compounds was also investigated. Yeast alcohol dehydrogenase was selected because this enzyme has an SH group essential to catalytic activity, as HPO lyase does, and it has been reported that yeast alcohol dehydrogenase is readily inactivated irreversibly by exposure to active oxygen formed by mixed-function oxidation reaction (Fucci et al., 1983). Under the conditions employed here, HPO lyase lost 75% of the initial activity during a 10-min incubation with LA 13-HPO. A positional isomer of LA 13-HPO, LA 9-HPO, also inactivated HPO lyase. LA 13-hydroxide caused little inactivation, which indicated that the HPO group was needed to inactivate HPO lyase. Matsushita et al. (1970) reported that binding of fatty acid HPO to some enzyme's hydrophobic regions, rather than a chemical reaction, could have been responsible for modification of the activity. Because HPO lyase was only slightly inactivated by LA 13-hydroxide, which is thought to have almost the same hydrophobic character as the corrresponding HPO, it was suggested that binding of fatty acid HPO to the hydrophobic region of HPO lyase was not responsible for the inactivation. Organic HPOs, such as *tert*-butyl and cumene HPOs, had substantially no effect on the activity of HPO lyase even if these HPOs were used at the higher concentration. Furthermore, H_2O_2 also had no effect. Yeast alcohol dehydrogenase was also inactivated by LA 13-HPO and lost 75% of the initial activity during a 10-min incubation. Apart from the results obtained with HPO lyase, the dehydrogenase lost over 90% of the initial activity by either *tert*-butyl or cumene HPOs. Hydrogen peroxide also appreciably inactivated the dehydrogenase.

Fatty acid HPO per se is thought not to be so reactive as to destroy an amino acid residue in a protein. It is suggested that fatty acid HPO is converted into a highly reactive species such as peroxy or alkoxy radical in inactivating HPO lyase. The specificity of inactivation of HPO lyase indicates that HPO lyase specifically introduces fatty acid HPO into a highly oxidizable region near the reaction center and converts the bound HPO to a highly reactive species at the restricted site. Because fatty acid HPOs are natural substrates for HPO lyase, it is reasonable to expect the existence of a specific recognition mechanism for fatty acid HPOs, while the mechanism of activation of fatty acid HPO is still unknown. Because LA 9-HPO which is scarcely catalyzed by HPO lyase also inactivates the enzyme (Table I), the possibility that fatty acid HPO is activated by the normal catalytic cycle forming *n*-hexanal and 12-oxo-9(*Z*)-dodecenoic acid from LA 13-HPO is not tenable. The fact that alcohol dehydrogenase was inactivated nonspecifically by various HPOs suggests that a residue susceptible to attack by reactive oxygen species is exposed on the surface of the enzyme.

Reagents listed in Table II were co-incubated with HPO lyase and LA 13-HPO. The reduced form of glutathione had no effect on the inactivation, although this reagent was effective in the efficient extraction of HPO lyase activity from tea leaves (Matsui et al., 1991). Glutathione may be effective for extraction because it protects HPO lyase in crude homogenate by decomposing endogenous HPOs via glutathione reductase (or related enzyme) activity (Schmidt and Kunert, 1986), while dithiothreitol substantially protected HPO lyase from the inactivation. As previously reported with HPO lyases from various plant tissues (Vick and Zimmerman, 1976; Phillips and Galliard, 1978; Schreier and Lorenz, 1982; Matsui et al., 1989, 1991), participation of an SH group in the reaction of this enzyme is suggested. Protection by dithiothreitol suggests that destruction of an essential SH group in HPO lyase at least partly contributes to the inactivation. Organic antioxidants such as α-tocopherol, nordihydroguaiaretic acid, butylated hydroxyanisole, and butylated hydroxytoluene also protected. These organic antioxidants have inhibitory effects on HPO lyase activity (Table II; Matsui et al., 1991) and are also believed to scavenge radical species originated from fatty acid or the other organic HPOs (Hal-

Table II. Protection against Inactivation of HPO Lyase[a]

reagent added	HPO	residual act., units/mg	rel residual act., % without HPO
none	−	18.9 ± 0.52 (100.0)[b]	
	+	4.8 ± 0.53	25.2 ± 2.81
5 mM glutathione	−	18.0 ± 0.81 (95.2)	
	+	4.9 ± 0.49	27.1 ± 2.74
5 mM dithiothreitol	−	19.2 ± 0.77 (101.6)	
	+	9.5 ± 1.13	50.0 ± 5.89
0.2 mM α-tocopherol	−	14.8 ± 0.32 (78.3)	
	+	10.7 ± 0.53	72.6 ± 3.57
0.2 mM nordihydro-guaiaretic acid	−	11.9 ± 0.26 (63.0)	
	+	6.6 ± 0.17	55.4 ± 1.39
0.5 mM butylated hydroxyanisole	−	14.6 ± 0.24 (77.2)	
	+	10.1 ± 0.17	68.6 ± 1.13
0.5 mM butylated hydroxytoluene	−	17.5 ± 1.41 (92.6)	
	+	11.5 ± 0.64	65.4 ± 3.67
1 mM DETAPAC[c]	−	18.0 ± 0.37 (95.2)	
	+	4.7 ± 0.29	26.3 ± 1.60
50 mM mannitol	−	18.9 ± 0.80 (100.0)	
	+	4.7 ± 0.14	25.0 ± 0.72
2.5% ethanol	−	18.4 ± 0.79 (97.4)	
	+	6.5 ± 0.52	35.3 ± 2.80
5% ethanol	−	17.0 ± 0.35 (89.9)	
	+	9.8 ± 0.50	57.9 ± 2.94
0.1 mM LA 13-hydroxide	−	10.6 ± 0.73 (56.1)	
	+	5.1 ± 0.37	48.0 ± 3.53

[a] HPO lyase (46.4 nM) was preincubated with or without 40 μM LA 13-HPO in 50 mM MES-KOH (pH 5.5) containing each reagent for 10 min at 25 °C. Residual activity was measured by diluting the preincubated mixture 10-fold with 32 μM LA 13-HPO in 50 mM MES-KOH (pH 5.5). Values are means ± SD of three repeats. [b] HPO lyase activities in the presence of each reagent (after 10-fold dilution) are shown as a relative activity (%). [c] DETAPAC, diethylenepentaacetic acid.

liwell and Gutteridge, 1985). Ethanol also showed protecting ability, although a relatively high concentration was needed. Ethanol is known to scavenge radicals, and it also slightly inhibited HPO lyase activity (Table II). A hydrophilic radical scavenger, mannitol, affected neither the activity nor the degree of inactivation. Furthermore, diethylenetriaminepentaacetic acid, which is known to suppress metal-catalyzed activation of HPOs, did not show any effect. As a whole, a hydrophobic radical scavenger that inhibited HPO lyase activity always protected it from inactivation by LA 13-HPO. But it must be noted that those having high inhibitory activity did not always have high protecting ability.

From these results, one probable inactivation mechanism was deduced; that is, fatty acid HPO specifically introduced near the reaction center of HPO lyase is converted to a hydrophobic radical species which in turn destroys an SH group essential to HPO lyase activity. To certify this hypothesis, we must detect formation of a radical species and identify the destroyed amino acid in HPO lyase.

The finding that fatty acid HPO potentially inactivates HPO lyase in tea leaves indicates that HPO lyase activity is finely regulated in vivo, although the precise physiological role of this enzyme has not been revealed. This finding may also offer some suggestions in the regulation of flavor of processed tea.

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LITERATURE CITED

Chamulitrat, W.; Takahashi, N.; Mason, R. P. Peroxy, alkoxyl, and carbon-centered radical formation from organic hydroperoxides by chloroperoxidase. *J. Biol. Chem.* **1989**, *264*, 7889–7899.

Dulley, J. R.; Grieve, P. A. Simple technique for eliminating interference by detergents in the Lowry method of protein determination. *Anal. Biochem.* **1975**, *64*, 136–141.

Fitzpatrick, P. F.; Villafranca, J. J. The mechanism of inactivation of dopamine β-hydroxylase by hydrazines. *J. Biol. Chem.* **1986**, *261*, 4510–4518.

Fucci, L.; Oliver, C. N.; Coon, M. J.; Stadtman, E. R. Inactivation of key metabolic enzymes by mixed-function oxidation reaction: Possible implication in protein turnover and ageing. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 1521–1525.

Gardner, H. W. Decomposition of linoleic acid hydroperoxides. Enzymic reactions compared with nonenzymic. *J. Agric. Food Chem.* **1975**, *23*, 129–136.

Gardner, H. W. Lipid hydroperoxide reactivity with proteins and amino acids: A review. *J. Agric. Food Chem.* **1979**, *27*, 220–229.

Gardner, H. W. Flavors and bitter tastes from oxidation of lipids by enzymes. In *Flavor Chemistry of Fats and Oils*; Min, D. B., Smouse, T. H., Eds.; American Oil Chemists' Society: Champaign, IL, 1985; Chapter 10.

Halliwell, B.; Gutteridge, J. M. C. Lipid peroxidation: A radical chain reaction. In *Free Radicals in Biology and Medicine*; Oxford University Press: Oxford, U.K., 1985; pp 139–189.

Hatanaka, A.; Kajiwara, T.; Sekiya, J.; Inouye, S. Solubilization and properties of the enzyme-cleaving 13-L-hydroperoxylinolenic acid in tea leaves. *Phytochemistry* **1982**, *21*, 13–17.

Kajiwara, T.; Sekiya, J.; Asano, M.; Hatanaka, A. Enantioselectivity of enzymatic cleavage reaction of 13-hydroperoxylinolenic acid to C_6-aldehyde and C_{12}-oxo-acid in tea chloroplasts. *Agric. Biol. Chem.* **1982**, *46*, 3087–3088.

Little, C.; O'Brien, P. J. The effectiveness of a lipid peroxide in oxidizing protein and non-protein thiols. *Biochem. J.* **1968**, *106*, 419–423.

Matsui, K.; Shibata, Y.; Kajiwara, T.; Hatanaka, A. Separation of 13- and 9-hydroperoxide lyase activities in cotyledons of cucumber seedlings. *Z. Naturforsch.* **1989**, *44C*, 883–885.

Matsui, K.; Toyota, H.; Kajiwara, T.; Kakuno, T.; Hatanaka, A. Fatty acid hydroperoxide cleaving enzyme, hydroperoxide lyase, from tea leaves. *Phytochemistry* **1991**, *30*, 2109–2113.

Matsushita, S.; Kobayashi, M.; Nitta, Y. Inactivation of enzymes by linoleic acid hydroperoxides and linoleic acid. *Agric. Biol. Chem.* **1970**, *34*, 817–824.

Phillips, D. R.; Galliard, T. Flavour biogenesis: Partial purification and properties of a fatty acid hydroperoxide cleaving enzyme from fruits of cucumber. *Phytochemistry* **1978**, *17*, 353–358.

Pietruszko, R.; Crawford, K.; Lester, D. Comparison of substrate specificity of alcohol dehydrogenases from human liver, horse liver, and yeast towards saturated and 2-enoic alcohols and aldehydes. *Arch. Biochem. Biophys.* **1973**, *159*, 50–60.

Schmidt, A.; Kunert, K. J. Lipid peroxidation in higher plants: The role of glutathione reductase. *Plant Physiol.* **1986**, *82*, 700–702.

Schreier, P.; Lorenz, G. Separation, partial purification and characterization of a fatty acid hydroperoxide cleaving enzyme from apple and tomato fruits. *Z. Naturforsch.* **1982**, *37C*, 165–173.

Vick, B. A.; Zimmermann, D. C. Lipoxygenase and hydroperoxide lyase in germinating watermelon seedlings. *Plant Physiol.* **1976**, *57*, 780–788.

Wurzenburger, M.; Grosch, W. The formation of 1-octen-3-ol from the 10-hydroperoxide isomer of linoleic acid by a hydroperoxide lyase in mushrooms (*Psalliota bispora*). *Biochim. Biophys. Acta* **1984**, *794*, 25–30.

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