Plant Cell Physiol. 26(7): 1291–1301 (1985)

Chlorophyll Metabolism in Higher Plants VI. Involvement of Peroxidase in Chlorophyll Degradation

Misako Kato and Seki Shimizu

Department of Biology, Faculty of Science, Ochanomizu University, Bunkyo-ku, Tokyo 112, Japan

The following phenolics were found to be essential for peroxidase-dependent chlorophyll bleaching: 2,4-dichlorophenol (DCP), *p*-coumaric acid (HCA), phenol, *p*-hydroxyphenylacetic acid, *p*-hydroxybenzoic acid, *p*-hydroxyacetophenone, resorcinol and umbelliferone. Most of them are monophenols with electron-attracting groups at the *p*-position. The short-lived radicals generated by horseradish peroxidase (HRP)-phenolics-H_2O_2 reaction might be involved in this reaction. Tobacco leaf enzyme preparation with peroxidase activity for guaiacol could also degrade chlorophyll with such phenolics. In addition, tobacco leaf methanol extract could substitute for chlorophyll bleaching as an electron donor in the absence of phenolics. In place of free H_2O_2, the glycolate-glycolate oxidase (GOX) system could degrade chlorophyll in [peroxidase+phenolics]-dependent bleaching.

This chlorophyll bleaching system was inhibited by peroxidase inhibitors, radical scavengers, reducing reagents, and carotenoids. Ascorbate and glutathione stopped chlorophyll bleaching with GSSG reductase and NADPH. The role of ascorbate and glutathione in peroxidase activity for controlling the chlorophyll degradation rate is discussed.

Key words: Chlorophyll bleaching — Free radical — Hydrogen peroxide — *Nicotiana tabacum* — Peroxidase — Phenolics.

Chlorophyll degradation is the most remarkable phenomenon occurring during leaf senescence and fruit maturation, but the intermediate steps in its biodegradation are still obscure. Though some chlorophyll degradation in leaves may result from photooxidation of the pigment, the fact that mature leaves lose chlorophyll in the dark indicates that degradation in vivo is at least partially enzymatic. Low temperatures and anaerobic conditions during incubation, boiling or freezing prior to incubation, and desiccation of leaves greatly reduce chlorophyll loss. The enzyme(s) responsible for chlorophyll degradation per se have not been clarified and the existing evidence is circumstantial.

Three possible enzymatic degradative pathways of chlorophyll bleaching in vitro have been reported. (i) Chlorophyllase (EC 3.1.1.14), found in many plant materials, hydrolyzes chlorophyll to chlorophyllide and phytol (Kuroki et al. 1981, Purvis and Barmore 1981). However, as the absorption spectrum of chlorophyllide is similar to that of chlorophyll, the decrease in the green color of the reaction mixture cannot be explained by chlorophyllase action alone. (ii) Lipoxygenase (EC 1.13.11.12) mediates polyunsaturated fatty acid oxidation and produces

Abbreviations: DCP, 2,4-dichlorophenol; DTT, dithiothreitol; DMSO, dimethyl sulfoxide; GOX, glycolate oxidase; HCA, hydroxycinnamic acid (*p*-coumaric acid); HRP, horseradish peroxidase; SHAM, salicylhydroxamic acid.

free radicals of fatty acid, which react with chlorophyll and oxidize it (Holden 1970, Sakai-Imamura 1975, Hildebrand and Hymowitz 1982, Klein et al. 1984). However, this enzyme is mainly present in non-green tissues (i.e., seeds and tubers) (Eriksson and Svensson 1970, Galliard and Phillips 1971) and, it is found exceptionally in young wheat leaves (Guss et al. 1968, Douillard and Bergeron 1981). (iii) Peroxidase (EC 1.11.1.7), predominantly found in plant materials, bleaches chlorophyll in the presence of H_2O_2 and certain phenolics—2,4-dichlorophenol (Matile 1980, Huff 1981, Martinoia et al. 1982) and resorcinol (Huff 1981).

The nature of the inducer(s) which starts senescence in plant cells is not yet understood. Among the various possibilities discussed, the free-radical theory has recently been attracting attention. We tried to elucidate the detailed characteristics of peroxidative chlorophyll degradation in vitro, and discuss here the participation of this degradative system in vivo.

Materials and Methods

Plant material—Tobacco plants (*Nicotiana tabacum* cv BY-2) were grown in a greenhouse. Leaves were harvested after 2 months.

Tobacco enzyme preparation—Acetone powder was prepared from tobacco leaves and extracted with 50 mM K-phosphate buffer, pH 7.0, containing 0.05% Triton X-100. The extracts were filtrated, centrifuged (12,000×*g* for 15 min), and dialyzed against 50 mM K-phosphate buffer, pH 7.0, for 24 hours. After concentration by ultrafiltration (Amicon, UM-10), each sample was applied to a Sephacryl S-200 column (2×90 cm) which had been equilibrated and eluted with the same buffer. The active fractions with peroxidative activity for guaiacol were pooled and used as the enzyme preparation.

Tobacco extracts—Tobacco leaves were extracted in 70% methanol containing 1% HCl. The extract was concentrated and subjected to gel filtration on a Sephadex LH-20 column (2.5×90 cm) equilibrated and eluted with 70% methanol. The fractions were monitored spectrophotometrically at 280 nm and those eluted after the void volume were pooled and used as a tobacco extract.

Chlorophyll a *and carotenoids*—Chlorophyll *a* was prepared according to Strain (1954) and solubilized in 5% Triton X-100, then used as a substrate for the bleaching assay.

Carotenoids were extracted and purified according to the method of Strain and Svec (1969).

Chlorophyll bleaching—The standard assay mixture contained 50 mM acetate buffer, pH 5.6, 40 μM phenolics, 2.5 units horse-radish peroxidase (Grade II, Bohringer) or tobacco enzyme preparation, 14.4 μM chlorophyll *a*, and 15 μM H_2O_2 in a final assay volume of 3.0 ml. The final Triton X-100 concentration was 0.17%. The reaction was started by adding H_2O_2 and followed spectrophotometrically at 665 nm.

Oxidation of phenolics—The oxidation of phenolics was detected by the absorbance change in a reaction mixture as described above except for chlorophyll. When HCA was used as a phenolic substance, a decrease of 289 nm, the absorption maximum of HCA in H_2O, was measured as the criterion of the peroxidase reaction.

Glycolate oxidase—Glycolate oxidase activity was determined according to the method of Brennan et al. (1979) with a slight modification. The reaction mixture contained the same components as the chlorophyll bleaching assay, 0.8 unit glycolate oxidase (Sigma) and 3.3 mM phenylhydrazine hydrochloride in place of chlorophyll. The formation of glyoxylate phenylhydrazone was followed spectrophotometrically at 324 nm.

All enzymatic reactions were carried out at 25°C.

Results

DCP-dependent peroxidative chlorophyll bleaching—In order to verify the results of initial studies indicating that HRP-H_2O_2 catalyzes chlorophyll bleaching in the presence of DCP (Matile 1980, Huff 1981), the reaction was started by adding H_2O_2 and/or DCP to the reaction mixture containing chlorophyll *a* and peroxidase. As shown in Fig. 1, in the absence of DCP and H_2O_2, no decrease at 665 nm was observed. Only in the presence of the substances, did the decrease start. The bleaching rate was in proportion to the DCP concentration up to 0.3 mM.

Chlorophyll *a* was subsequently added to the reaction mixture containing peroxidase and HCA in which the reaction had been started by adding H_2O_2 (Fig. 2). Later, chlorophyll was added and a smaller amount of chlorophyll was bleached. The result suggested that a short-lived intermediate(s) was formed in the [peroxidase-H_2O_2-HCA] reaction and it reacted with chlorophyll *a*. The same effect was observed with the addition of umbelliferone. In place of chlorophyll, HCA was added after the reaction had been started, and bleaching took place regardless of the time of addition.

Effects of phenolic compounds in chlorophyll bleaching—As shown in Fig. 1, DCP was effective in peroxidative chlorophyll degradation. However, it has not been found in plant materials. We examined the effectiveness of various natural phenolic compounds—phenols, phenolic acids, coumarines and flavonoids—as cofactors in peroxidative chlorophyll degradation (Table 1). For this purpose, phenolics were dissolved in DMSO and added to the reaction mixture to give a final DMSO concentration of 0.4%. At this concentration, DMSO had no effect on chlorophyll bleaching.

Many phenolics were oxidized as electron donors for peroxidase, but not all of them acted as chlorophyll bleaching cofactors. The following seven compounds were effective: phenol, *p*-hydroxybenzoic acid, HCA, resorcinol, *p*-hydroxyphenylacetic acid, *p*-hydroxyacetophenone and umbelliferone. These compounds were all monophenol derivatives with *p*-position substitution.

Fig. 3 gives the representative data for the chlorophyll bleaching rate of these compounds. The high initial bleaching rate did not mean a large amount of bleached chlorophyll. For

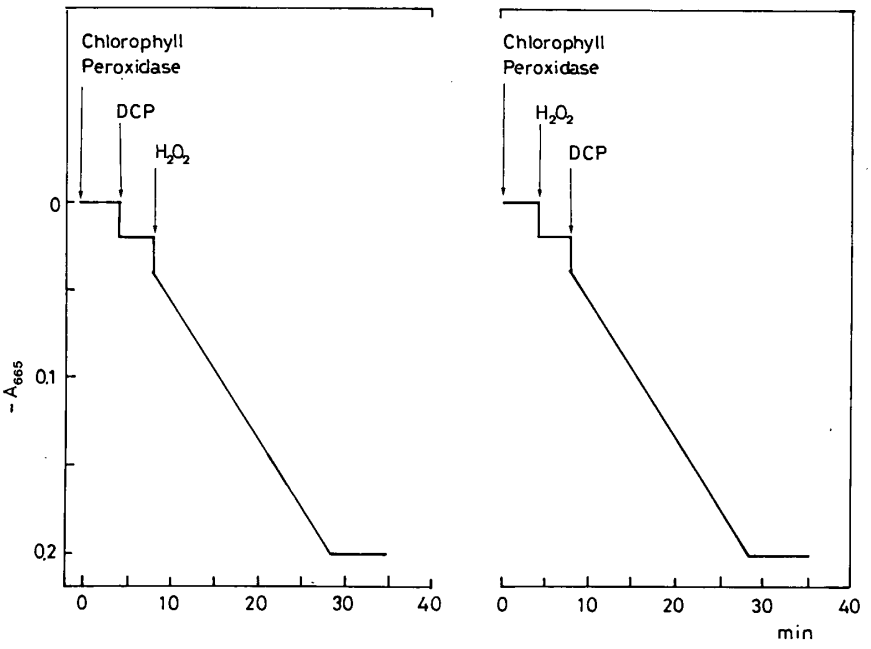


Fig. 1 Dependence of peroxidase-catalyzed chlorophyll bleaching on the presence of DCP.

Table 1 Effects of phenolic and other compounds on chlorophyll bleaching

	Oxidation	Chlorophyll bleaching
Monophenols and derivatives		
Phenol	+	++
p-Hydroxybenzoic acid	+	+
o-Hydroxyphenylacetic acid	+	−
m-Hydroxyphenylacetic acid	+	−
p-Hydroxyphenylacetic acid	+	++
p-Hydroxyacetophenone	+	+
o-Coumaric acid	+	−
p-Coumaric acid	+	+++
2,4-Dichlorophenol	+	+++
o-Diphenols and derivatives		
Catechol	+	−
o-Methoxyphenol (Guaiacol)	+	−
p-Methoxyphenol	+	−
Vanillic acid	+	−
Ferulic acid	+	−
Caffeic acid	+	−
Chlorogenic acid	+	−
m-Diphenols and derivatives		
Resorcinol	+	+++
3,5-Dihydroxybenzoic acid	+	−
2,6-Dihydroxybenzoic acid	+	−
3,4-Dihydroxybenzoic acid (Protocatechuic acid)	+	−
p-Diphenols and derivatives		
Hydroquinone	+	−
2,5-Dihydroxybenzoic acid	+	−
Coumarines and derivatives		
Scopoletin	+	−
Scopolin	−	−
Umbelliferone	+	+++
Esculin	+	−
Flavonoids and derivatives		
Flavone	−	−
Quercetin	+	−
Myricetin	+	−
Kaempferol	+	−
Rutin	+	−
Others		
Quinic acid	−	−
Abscisic acid	−	−
Ascorbic acid	+	−

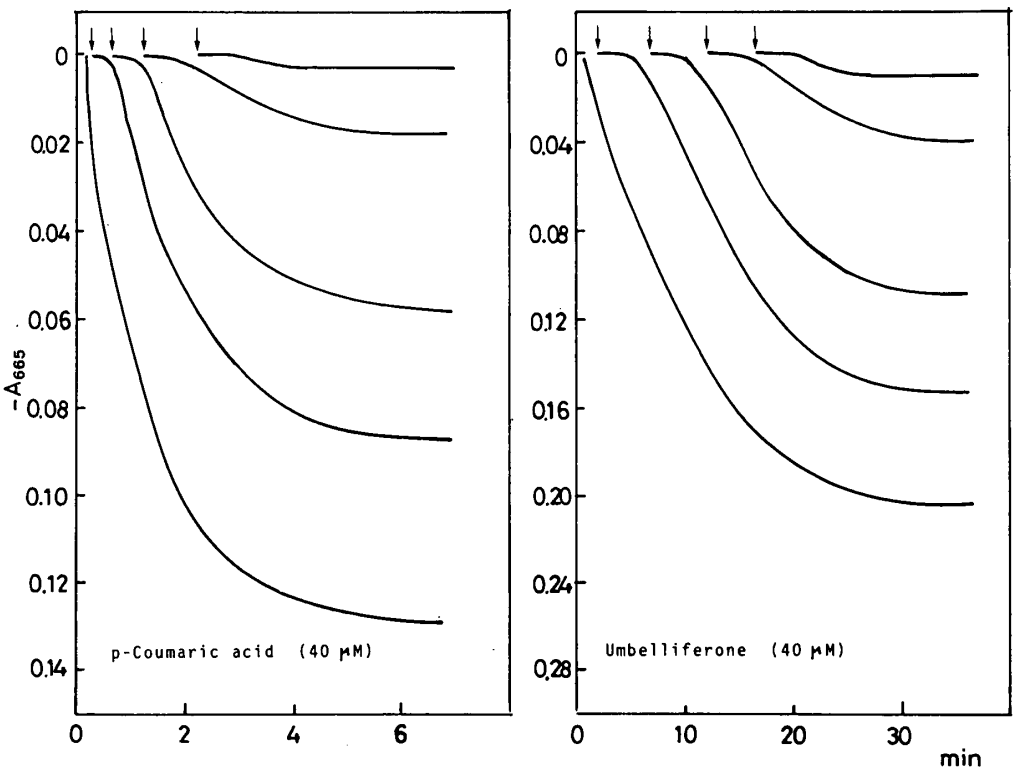


Fig. 2 Effects of time of addition on chlorophyll bleaching reaction. Chlorophyll was added to the HRP-phenolics-H_2O_2 system as shown by arrows.

example, the initial rate with HCA was 5.5 times as fast as that with resorcinol, but the total bleaching was only about half.

Inhibition for chlorophyll bleaching—Table 2 shows the inhibitory effect of some compounds on

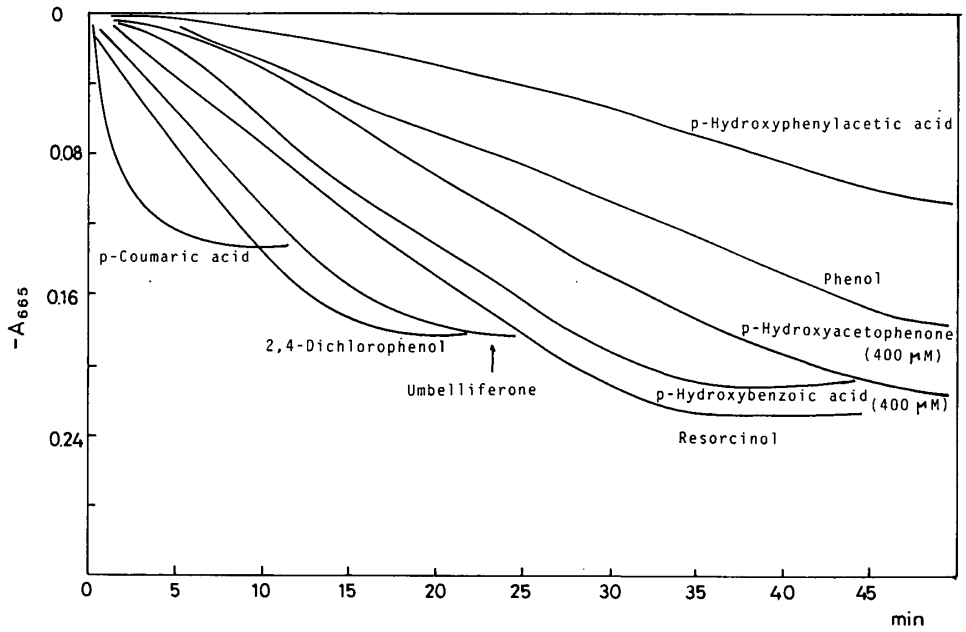


Fig. 3 Rates of chlorophyll bleaching by various phenolic compounds. Each reaction mixture contained 50 mM acetate buffer (pH 5.6), 14.4 μM chlorophyll, 40 μM phenolics, 2.5 units HRP, and 15 μM H_2O_2 in 3.0 ml, but the concentration of both *p*-hydroxybenzoic acid and *p*-hydroxyacetophenone was 400 μM.

Table 2 Effects of various compounds on chlorophyll bleaching

	Inhibition (%)	
	Chl bleaching	HCA destruction
Inhibitor		
KCN (4 mM)	100	100
NaN_3 (4 mM)	84.8	95.0
3-Amino-1,2,4-triazole (4 mM)	15.3	8.4
Radical scavenger		
α-Tocopherol (40 μM)	93.3	90.1
Triethylenediamine (4 mM)	68.7	9.1
n-Propyl gallate (0.4 mM)	100	100
SHAM (0.4 mM)	100	100
Tiron (0.3 mM)	100	100
(30 μM)	71.2	52.2
Reducing reagent		
DTT (1 mM)	37.0	—
Ascorbate (0.3 mM)	100	—
(30 μM)	100	—
GSH (3 mM)	4.3[a]	—
Carotenoid		
β-Carotene (15 μg/3 ml)	32.3	—
Lutein (15 μg/3 ml)	48.3	—
Violaxanthin (15 μg/3 ml)	24.2	—
Neoxanthin (15 μg/3 ml)	74.2	—

[a] GSH shows a low value at pH 5.6 but displays a higher value at pH 7.8.

peroxidative chlorophyll bleaching. KCN and NaN_3 inhibited chlorophyll bleaching as well as the peroxidase reaction. The catalase inhibitor 3-amino-1,2,4-triazole had a minor effect on both reactions. The free radical scavenger triethylenediamine (Fahrenholtz et al. 1974, Pooler et al. 1979) inhibited 70% of chlorophyll bleaching, while peroxidase activity was inhibited by only 10%. *n*-Propyl gallate, SHAM (Siedow and Girvin 1980) and Tiron (Bousquet and Thimann 1984) effectively inhibited chlorophyll bleaching and peroxidative destruction of HCA. Of the four carotenoids, neoxanthin was the most effective. Reducing reagents also inhibited chlorophyll bleaching. The effect of glutathione was small, and the inhibition depended on pH, increasing with higher pH. The effect of tobacco enzyme against these inhibitors was same as that of HRP.

The effects of ascorbate and GSH on chlorophyll bleaching were investigated further (Fig. 4). Ascorbate at 3 mM inhibited chlorophyll bleaching completely, and 100 μM caused a 2-min delay. In the presence of 12.5 μM GSH, the rate of chlorophyll bleaching decreased over the range of the reaction. This inhibition increased when GSSG reductase and NADPH were added, making reproduction of GSH possible. Furthermore, when ascorbate was added to this reaction mixture, no chlorophyll bleaching was observed.

Bleaching rate with H_2O_2-generating system—In the presence of phenolics, peroxidative chlorophyll bleaching needs H_2O_2 to start the reaction. Thus, the possibility of coupling between the H_2O_2-generating reaction and peroxidative chlorophyll bleaching was examined. For this purpose, the glycolate-glycolate oxidase (GOX) reaction was selected as the H_2O_2-generating

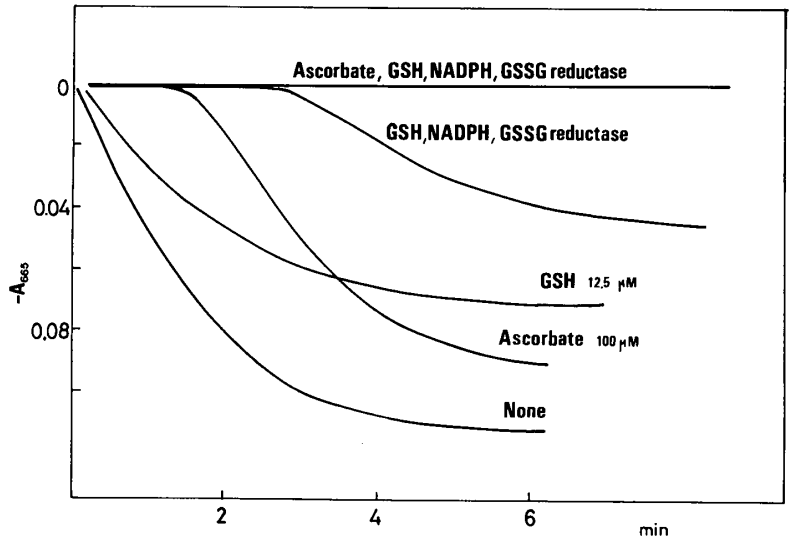


Fig. 4 Effects of ascorbate and glutathione on chlorophyll bleaching. Each reaction mixture contained 0.1 M phosphate buffer (pH 7.8), 14.4 μM chlorophyll, 40 μM HCA, 7.5 units HRP, and 150 μM H_2O_2 in 3.0 ml, with addition of 100 μM ascorbate, 12.5 μM GSH, 150 μM NADPH, and/or 0.15 unit GSSG reductase (Sigma, Type III).

reaction. Both chlorophyll bleaching rate and the GOX activity were proportional to the glycolate concentration (Fig. 5). When the reaction occurred in the absence of HCA, no chlorophyll bleaching took place.

Chlorophyll bleaching with tobacco extract—The aqueous crude extract of tobacco leaves could catalyze [DCP+H_2O_2]-dependent chlorophyll bleaching only after removal of interfering low-molecular-weight substances from the crude extract using Sephacryl S-200 gel filtration.

The concentrated methanol extract of tobacco leaves could be used as a cofactor for chlorophyll bleaching in place of DCP (Table 1).

Discussion

HRP is the most investigated peroxidase from plant sources, and its reaction is postulated to be as follows:

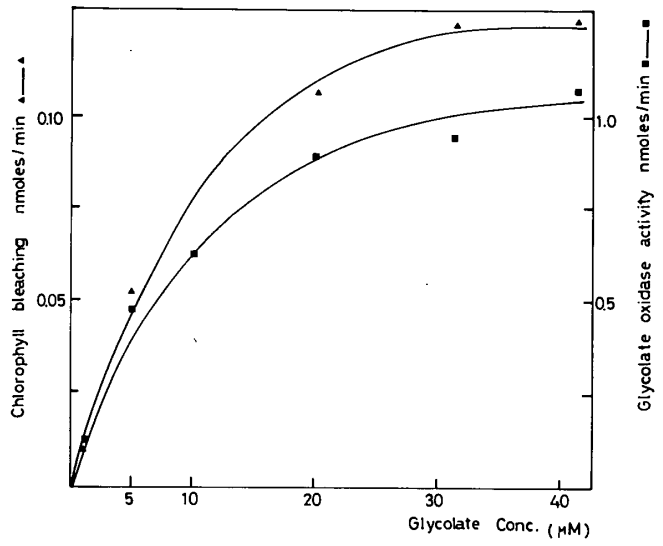


Fig. 5 Availability of H_2O_2 generated from glycolate-GOX reaction for chlorophyll bleaching. For experimental details see Materials and Methods. –▲–, chlorophyll bleaching rate, –■–, H_2O_2 generating rate.

$$HRP + H_2O_2 \longrightarrow HRP\text{-}I$$
$$HRP\text{-}I + AH \longrightarrow HRP\text{-}II + AH\cdot$$
$$HRP\text{-}II + AH \longrightarrow HRP + AH\cdot$$

AH_2 is an electron donor and gives a free radical expressed as AH· when the peroxidase reaction is carried out. AH· is converted into a stable form by non-enzymatic polymerization or is kept inactive when its reactivity is small. When chlorophyll was added after the HRP-phenolics system which had been started by the addition of H_2O_2, the initial bleaching rate and the final amount of bleached chlorophyll decreased with time. However, when phenolics were added late to the HRP-H_2O_2-chlorophyll system, the bleaching rate did not decrease. These observations lead to the conclusion that the free radicals produced from phenolics mediate chlorophyll destruction. The free radical had such high reactivity that it could be converted into other inactive forms in a short period and thus could not be responsible for chlorophyll bleaching when the peroxidase reaction was started prior to the addition of chlorophyll. Fig. 6 shows the constitutional formulas of the phenolic compounds tested; each has an electron-attractive group at the *p*-position.

The free radicals derived from these phenolics are postulated to have high reactivity due to the disproportion of electrons in the molecule. However, this electron attractivity is not always proportional to the chlorophyll bleaching activity. For example, *p*-hydroxyphenylacetic acid and *p*-hydroxybenzoic acid with highly electron-attractive groups are less effective. They may be so highly active that they react with other compounds. Furthermore, steric hindrance due to substituted groups at positions other than *para* causes chlorophyll bleaching only to a limited extent.

The question yet to be answered is whether this phenolics dependent-peroxidative chlorophyll bleaching is involved in the senescent process of living plants. In the presence of HCA, chloro-

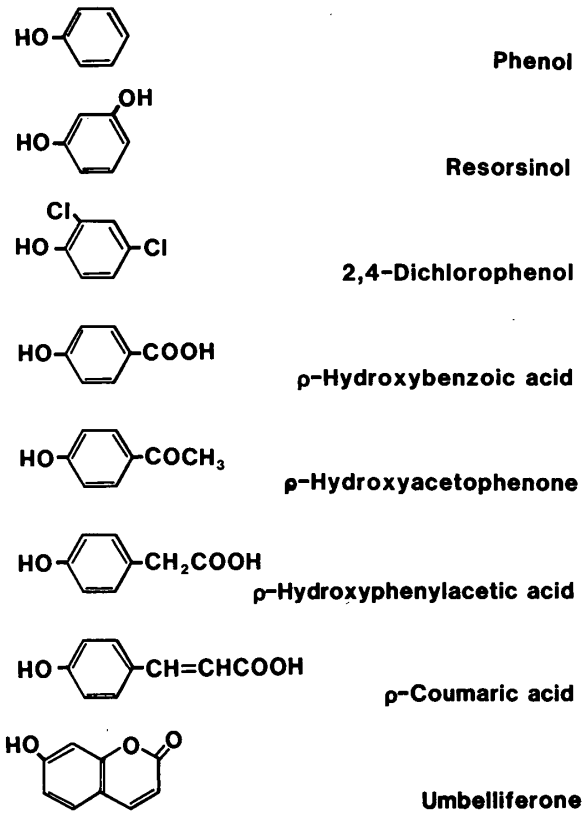
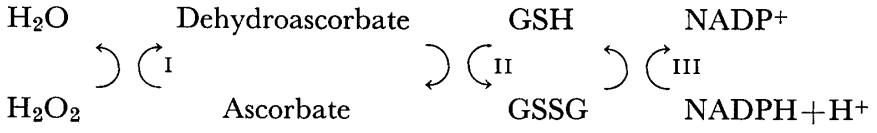


Fig. 6 Formulas of phenolic compounds possibly acting as electron donors in peroxidase-catalyzed chlorophyll bleaching.

phyll bleaching was carried out with the gel-filtrated tobacco protein which has peroxidase activity. Furthermore, the partially purified methanol extract of tobacco leaves was also effective in the above system in place of HCA. The isolation and characterization of the responsible substances in this extract are in progress.

Hydrogen peroxide, the other factor in this peroxidative chlorophyll bleaching, is produced commonly in photosynthesis (Mehler 1951, Robinson et al. 1980, Steiger and Beck 1981) or photorespiration (Huang 1982). Recently, the values of H_2O_2 evolution were reported; in broken chloroplasts, 15–35 μmoles·mg $Chl^{-1}\cdot h^{-1}$ (Robinson et al. 1980) and 5.5 μmoles·mg $Chl^{-1}\cdot h^{-1}$ (Steiger and Beck 1981); in intact chloroplasts, 0.85 μmoles·mg $Chl^{-1}\cdot h^{-1}$. Assuming that the osmotic volume is 21 μl·mg Chl^{-1} (Foyer and Halliwell 1976), the latter value shows that H_2O_2 concentration increases at the rate of 0.67 mM/min when there is no scavenging of H_2O_2.

Foyer and Halliwell (1976) suggested that the cycle constituted by glutathione and ascorbate plays a scavenger role in removing H_2O_2 as follows;



I Ascorbate peroxidase
II Dehydroascorbate reductase or a non-enzymatic process
III GSSG reductase

Glutathione peroxidase scavenges H_2O_2 in microsomes of animals (Chiu et al. 1976, Eklow et al. 1984), but is absent from plants (Jablonski and Anderson 1984). Ascorbate peroxidase differs from HRP in having a high affinity towards ascorbate, and is a stromal enzyme (Nakano and Asada 1981) inhibited by KCN, NaN_3, 8-hydroxyquinoline (Jablonski and Anderson 1982). The observation that the combination of ascorbate, GSH, GSSG reductase and NADPH could retard chlorophyll bleaching (Fig. 4) indicated that the ascorbate-GSH cycle might play a role in protecting chlorophyll against oxidative damage.

Phenolics-peroxidase-dependent chlorophyll bleaching might be a key to understanding chlorophyll degradation in senescent leaves. One of the most important questions concerns the subcellular organization of this process in living cells. Martinoia et al. (1982) reported that chloroplasts from barley mesophyll protoplasts contained peroxidative activity on chlorophyll bleaching. But they did not detect the peroxidase activity against guaiacol as an electron donor, and moreover detected the chlorophyll-bleaching activity in the presence of SDS. In the present investigation, we observed that the chlorophyll-protein complex prepared with SDS was bleached easily without peroxidase and/or H_2O_2. This detergent might be a potent activator for the peroxidase-catalyzed chlorophyll bleaching. We could not detect peroxidase activity with guaiacol in isolated chloroplasts and other workers have reported that the principal site of this enzyme was the vacuole (Boller and Kende 1979, Grob and Matile 1980, Thom et al. 1982). In younger plants, compartmentation prevents chlorophyll from contacting the peroxidase in the vacuole. As senescence advances, membrane lipid peroxidation causes membrane leakiness (Barker and Thompson 1980, Dhindsa et al. 1981), and interaction between vacuoles and chloroplasts might take place (Peoples et al. 1980, Wittenbach et al. 1982). Another item to study is the seasonal change of the protective mechanism (i.e. levels of ascorbate, reduced glutathione, catalase and glutathione reductase) and its effect on foliar senescence. This work is now in progress.

We wish to thank Drs. S. Kubo and T. Fujimori of Central Research Institute, Japan Tobacco Inc., for supplying scopolin and for their kind advice in this study. We also greatly appreciate the help of Prof. K. Maeda, Ochanomizu University, for her valuable suggestions on the chemical theory of free radicals.

References

Barber, R. F. and J. E. Thompson (1980) Senescence-dependent increase in the permeability of liposome prepared from bean cotyledone membranes. *J. Exp. Bot.* 31: 1305–1313.

Boller, T. and H. Kende (1979) Hydrolytic enzymes in the central vacuole of plant cells. *Plant Physiol.* 63: 1123–1132.

Bousquet, J.-F. and K. V. Thimann (1984) Lipid peroxidation forms ethylene from 1-aminocyclopropane-1-carboxylic acid and may operate in leaf senescence. *Proc. Natl. Acad. Sci. USA* 81: 1724–1727.

Brennan, T., A. Rychter and C. Frenkel (1979) Activity of enzymes involved in the turnover of hydrogen peroxide during fruit senescence. *Bot. Gaz.* 140: 384–388.

Chiu, D. Y., F. H. Stults and A. Tappel (1976) Purification and properties of rat lung soluble glutathione peroxidase. *Biochim. Biophys. Acta* 445: 558–566.

Dhindsa, R. S., P. Plumb-Dhindsa and T. A. Thorpe (1981) Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J. Exp. Bot.* 32: 93–101.

Douillard, R. and E. Bergeron (1981) Lipoxygenase activities of young wheat leaves. *Physiol. Plant.* 51: 335–338.

Eklow, L., P. Moldeus and S. Ohrenius (1984) Oxidation of glutathione during hydroperoxide metabolism—A study using isolated hepatocytes and the glutathione reductase inhibitor 1,3-bis(2-chloroethyl)-1-nitrosourea. *Eur. J. Biochem.* 138: 459–463.

Eriksson, C. E. and S. G. Svensson (1970) Lipoxygenase from peas, purification and properties of the enzyme. *Biochim. Biophys. Acta* 198: 449–459.

Fahrenholtz, S. R., F. H. Doleiden, A. M. Trozzolo and A. A. Lamola (1974) On the quenching of the singlet oxygen by α-tocopherol. *Photochem. Photobiol.* 20: 505–509.

Foyer, C. H. and B. Halliwell (1976) The presence of glutathione and glutathione reductase in chloroplasts; A proposed role in ascorbic acid metabolism. *Planta* 133: 21–25.

Galliard, T. and D. R. Phillips (1971) Lipoxygenase from potato tubers. *Biochem. J.* 124: 431–438.

Grob, K. and Ph. Matile (1980) Compartmentation of ascorbic acid in vacuoles of horseradish root cells. Note on vacuolar peroxidase. *Z. Pflanzenphysiol.* 98: 235–243.

Guss, P. L., V. Macko, T. Richardson and M. A. Stahmann (1968) Lipoxygenase in early growth of wheat. *Plant Cell Physiol.* 9: 415–422.

Hildebrand, D. F. and T. Hymowitz (1982) Carotene and chlorophyll bleaching by soybeans with and without seed lipoxygenase-1. *J. Agric. Food Chem.* 30: 705–708.

Holden, M. (1961) The breakdown of chlorophyll by chlorophyllase. *Biochem. J.* 78: 359–364.

Huang, A. H. C. (1982) Metabolism in plant peroxisomes. *In* Recent Advances in Phytochemistry. Vol. 16. Edited by L. L. Creasy and G. Hrazdina. pp. 85–123. Plenum Press, New York.

Huff, A. (1982) Peroxidase-catalysed oxidation of chlorophyll by hydrogen peroxide. *Phytochemistry* 21: 261–265.

Jablonski, P. P. and J. W. Anderson (1982) Light-dependent reduction of hydrogen peroxide by ruptured pea chloroplast. *Plant Physiol.* 69: 1407–1413.

Jablonski, P. P. and J. W. Anderson (1984) Role of a flavonoid in the peroxide-dependent oxidation of glutathione catalysed by pea extracts. *Phytochemistry* 23: 1865–1869.

Klein, B. P., S. Grossman, D. King, B. S. Cohen and A. Pinsky (1984) Pigment bleaching, carbonyl production and antioxidant effects during the anaerobic lipoxygenase reaction. *Biochim. Biophys. Acta* 793: 72–79.

Kuroki, M., Y. Shioi and T. Sasa (1981) Purification and properties of soluble chlorophyllase from tea leaf sprouts. *Plant Cell Physiol.* 22: 717–725.

Martinoia, E., M. J. Dalling and Ph. Matile (1982) Catabolism of chlorophyll: Demonstration of chloroplast-localized peroxidative and oxidative activities. *Z. Pflanzenphysiol.* 107: 269–279.

Matile, Ph. (1980) Catabolism of chlorophyll: Involvement of peroxidase? *Z. Pflanzenphysiol.* 99: 475–478.

Mehler, A. H. (1951) Studies on reactions of illuminated chloroplast I. Mechanism of the reduction of oxygen and other Hill reagents. *Arch. Biochem. Biophys.* 31: 65–77.

Nakano, Y. and K. Asada (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* 22: 867–880.

Peoples, M. B., V. C. Beilharz, S. P. Waters, R. J. Simpson and M. J. Dalling (1980) Nitrogen redistribution during grain growth in wheat (*Triticum aestivum* L.) II. Chloroplast senescence and the degradation of ribulose-1,5-biphosphate carboxylase. *Planta* 149: 241–251.

Pooler, J. P. and D. P. Valenzeno (1979) The role of singlet oxygen in photooxidation of excitable cell membranes. *Photochem. Photobiol.* 68: 581–584.

Purvis, A. C. and C. R. Barmore (1981) Involvement of ethylene in chlorophyll degradation in peel of citrus fruits. *Plant Physiol.* 69: 854–856.

Robinson, J. M., M. G. Smith and M. Gibbs (1980) Influence of hydrogen peroxide upon carbon dioxide photoassimilation in the spinach chloroplast in an "intact" chloroplast preparation is a casual agent of the Warburg effect. *Plant Physiol.* 65: 755–759.

Sakai-Imamura, M. (1975) Fatty acid oxidation and chlorophyll bleaching. *Natl. Sci. Rep. Ochanomizu Univ.* 26: 109–125.

Siedow, J. N. and M. E. Girvin (1980) Alternative respiration pathway—Its role in seed respiration and its inhibition by propyl gallate. *Plant Physiol.* 65: 669–674.

Steiger, H.-M. and E. Beck (1981) Formation of hydrogen peroxide and oxygen dependence of photosynthetic CO_2 assimilation by intact chloroplasts. *Plant Cell Physiol.* 22: 561–576.

Strain, H. H. (1954) Oxidation and isomerization reactions of the chlorophylls in killed leaves. *Agric. Food Chem.* 2: 1222–1226.

Strain, H. H. and W. A. Svec (1969) *In* Advances in Chromatography. Vol. 8. Edited by J. C. Giddings and R. A. Keller. pp. 119–176.

Thom, M., A. Maretzki and E. Komor (1982) Vacuoles from sugar cane suspension cultures. I. Isolation and partial characterization. *Plant Physiol.* 69: 1315–1319.

Wittenbach, V. A., W. Lin and R. R. Hebert (1982) Vacuolar localization of proteases and degradation of chloroplasts in mesophyll protoplasts from senescing primary wheat leaves. *Plant Physiol.* 69: 98–102.

(Received January 28, 1985; Accepted July 23, 1985)