Planta (2009) 230:639–648
DOI 10.1007/s00425-009-0964-9

ORIGINAL ARTICLE

Evaluation of the toxicity of stress-related aldehydes to photosynthesis in chloroplasts

Jun'ichi Mano · Fumitaka Miyatake · Eiji Hiraoka · Masahiro Tamoi

Received: 14 January 2009 / Accepted: 18 May 2009 / Published online: 4 July 2009

Abstract Aldehydes produced under various environmental stresses can cause cellular injury in plants, but their toxicology in photosynthesis has been scarcely investigated. We here evaluated their effects on photosynthetic reactions in chloroplasts isolated from *Spinacia oleracea* L. leaves. Aldehydes that are known to stem from lipid peroxides inactivated the CO_2 photoreduction to various extents, while their corresponding alcohols and carboxylic acids did not affect photosynthesis. α,β-Unsaturated aldehydes (2-alkenals) showed greater inactivation than the saturated aliphatic aldehydes. The oxygenated short aldehydes malondialdehyde, methylglyoxal, glycolaldehyde and glyceraldehyde showed only weak toxicity to photosynthesis. Among tested 2-alkenals, 2-propenal (acrolein) was the most toxic, and then followed 4-hydroxy-(*E*)-2-nonenal and (*E*)-2-hexenal. While the CO_2-photoreduction was inactivated, envelope intactness and photosynthetic electron transport activity ($H_2O \rightarrow$ ferredoxin) were only slightly affected. In the acrolein-treated chloroplasts, the Calvin cycle enzymes phosphoribulokinase, glyceraldehyde-3-phosphate dehydrogenase, fructose-1,6-bisphophatase, sedoheptulose-1,7-bisphosphatase, aldolase, and Rubisco were irreversibly inactivated. Acrolein treatment caused a rapid drop of the glutathione pool, prior to the inactivation of photosynthesis. GSH exogenously added to chloroplasts suppressed the acrolein-induced inactivation of photosynthesis, but ascorbic acid did not show such a protective effect. Thus, lipid peroxide-derived 2-alkenals can inhibit photosynthesis by depleting GSH in chloroplasts and then inactivating multiple enzymes in the Calvin cycle.

Keywords Acrolein · 2-Hexenal · 4-Hydroxy-2-nonenal · Lipid peroxide · Oxidative stress · *Spinacia*

Abbreviations

AER	2-Alkenal reductase
Asc	Ascorbic acid
Chl	Chlorophyll
FBPase	Fructose-1,6-bisphosphatase
Fd	Ferredoxin
GAP	Glyceraldehyde 3-phosphate
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
HHE	4-Hydroxy-(*E*)-2-hexenal
HNE	4-Hydroxy-(*E*)-2-nonenal
LOOH	Lipid peroxide(s)
MDA	Malondialdehyde
PGA	3-Phosphoglyceric acid
PRK	Phosphoribulokinase
SBPase	Sedoheptulose-1,7-bisphosphatase

Introduction

Aldehydes occur as intermediates in various cellular pathways such as carbohydrate, amino acid, lipid and phenylpropanoid metabolisms. They can be formed also by lipid peroxidation,

J. Mano (✉)
Science Research Center, Yamaguchi University,
Yoshida 1677-1, Yamaguchi 753-8515, Japan
e-mail: mano@yamaguchi-u.ac.jp

F. Miyatake
Graduate School of Agriculture, Yamaguchi University,
Yoshida 1677-1, Yamaguchi 753-8515, Japan

E. Hiraoka
Faculty of Agriculture, Yamaguchi University,
Yoshida 1677-1, Yamaguchi 753-8515, Japan

M. Tamoi
Faculty of Agriculture, Kinki University,
3327-204 Nakamachi, Nara 631-8505, Japan

ascorbate autoxidation, or cytochrome P450s (O'Brien et al. 2005). Aldehydes are reactive molecules and have potential to modify proteins and nucleic acids (Burcham 1998; O'Brien et al. 2005). Due to this reactivity, they, at low concentrations, can act as signaling molecules for inducing stress defense genes (Alméras et al. 2003; Weber et al. 2004), while at higher concentrations they may exert cytotoxicity. In animal studies, aldehydes have been shown to cause mutation, cancer and cell-degenerating diseases (Comporti 1985; Esterbauer et al. 1991; Arlt et al. 2002; Nair et al. 2007).

In plants, there is increasing evidence for the toxicity of aldehydes in environmental and biotic stresses. Lipid peroxide (LOOH)-derived aldehydes, detected as thiobarbituric acid reactive substances, increased in association with biotic and abiotic stress-induced damages that were caused by bacterial infection (Muckenschnabel et al. 2001), ozone (Sakaki et al. 1983), chilling (Hodgson and Raison 1991), UV-B (Panagopoulos et al. 1992), heat (Mishra and Singhal 1992), intense light (Sharma and Singhal 1992), and drought (Moran et al. 1994). Such damage could be suppressed by aldehyde-scavenging enzymes when they were genetically enhanced in transgenic plants, as follows: Aldehyde reductase from *Medicago sativa* (alfalfa) overexpressed in *Nicotiana tabacum* (tobacco) improved their tolerance to drought stress (Oberschall et al. 2000), methyl viologen and UV-B (Hideg et al. 2003). Aldehyde dehydrogenase isozymes from *Arabidopsis thaliana* improved the tolerance of transgenic *A. thaliana* plants to NaCl, heavy metals, methyl viologen, and H_2O_2 (Sunkar et al. 2003). Vice versa, their knockout mutants were more sensitive to dehydration and salt than the wild type (Kotchoni et al. 2006). The observed protection was attributable to the scavenging of aldehydes because the level of thiobarbituric acid reactive substances was decreased by the overexpression of these enzymes and increased by the deficiency. Methylglyoxal, a by-product aldehyde in the triose-phosphate metabolism, is increased by NaCl stress (Yadav et al. 2005). Overexpression of the scavenging enzyme glyoxalase I in tobacco suppressed the increase in methylglyoxal (Yadav et al. 2005) and improved the tolerance to NaCl (Singla-Pareek et al. 2003). These results demonstrated a toxic action of methylglyoxal. A novel enzyme NADPH:2-alkenal reductase (AER; EC 1.3.1.74) found in *A. thaliana* (Mano et al. 2002) also improved the tolerance of transgenic tobaccos to intense light and to methyl viologen (Mano et al. 2005). This suggested that α,β-unsaturated aldehydes (2-alkenals) were produced on the photooxidative treatment and caused damage because AER specifically reduces the C–C double bond of a 2-alkenal to form a saturated aldehyde (Mano et al. 2002).

The targets of aldehyde toxicity in cells have been investigated mainly in animal cells. Several enzymes in the energy metabolism, including those in mitochondria (Chen et al. 1998; Humphries and Szweda 1998) are sensitive to 4-hydroxy-(*E*)-2-nonenal (HNE), one of the most reactive aldehydes produced from LOOH (Esterbauer et al. 1991). In plants also, mitochondrial enzymes are affected by HNE, as follows: respiratory reactions in pea mitochondria with various electron donors were inactivated by HNE, among which the glycine-dependent respiration was the most sensitive (Millar and Leaver 2000). The target site was the lipoate moiety of H-protein in the glycine decarboxylase complex and of other lipoate enzymes (Taylor et al. 2002). HNE also inactivated alternative oxidase (Winger et al. 2005). Recently, evidence has been provided for that the endogenously produced HNE modifies mitochondrial proteins in oxidative-stressed *A. thaliana* plants (Winger et al. 2007).

Chloroplast components would also be the potential targets of aldehydes. Chloroplasts produce reactive oxygen species such as singlet oxygen and superoxide radical in the vicinity or in the thylakoid membrane (Asada 2006). Considering that the thylakoid lipids are rich in polyunsaturated fatty acids, LOOH formation via these reactive oxygen species (Comporti 1985) is very likely, and from LOOH, many aldehydes such as malondialdehyde (MDA) can be formed by non-enzymatic mechanisms (Blée 1998). Chloroplasts have also enzymes to produce aldehydes; lipoxygenases catalyzing the oxygenation of polyunsaturated fatty acids to form LOOH, and hydroperoxide lyase to cleave it (Farmaki et al. 2007) to form C_6-aldehydes such as (*Z*)-3-hexenal and *n*-hexanal, typical 'green' volatile organic compounds (Matsui 2006). Yamauchi et al. (2008) very recently reported that in heat-stressed plants the chloroplast proteins OEC33 and LHCII were modified with MDA. This is direct evidence for the formation and action of MDA in chloroplasts. Furthermore, the phototolerance of the AER-overproducing tobaccos (Mano et al. 2005) implies that 2-alkenals also would damage chloroplast components.

In order to obtain new insights into the action of aldehydes in photosynthesis, we investigated the toxicity of various aldehydes to chloroplasts. Several kinds of aldehydes including MDA, methylglyoxal and HNE were compared. Using 2-propenal (acrolein), the most toxic aldehyde found, targets in chloroplasts were investigated.

Materials and methods

Chemicals

Acrolein, HNE and MDA were prepared by acid hydrolysis of acrolein diethyl acetal, HNE-diethylacetal (Alexis Biochemicals, care of Biolinks, Tokyo, Japan) and 1,1,3,3-tetramethoxypropane, respectively. 4-Hydroxy-(*E*)-2-hexenal

(HHE) was purchased from Cayman Chemical (Ann Arbor, MI, USA). Other aldehydes were of reagent grade. Glyceraldehyde 3-phosphate (GAP), ribose 5-phosphate, ribulose 5-phosphate and ribulose 1,5-bisphosphate were from Sigma Japan (Tokyo). Sedoheptulose 1,7-bisphosphate was enzymatically synthesized (Tamoi et al. 2005). Sodium [^{14}C]-bicarbonate was from PerkinElmer Japan (Tokyo).

Preparation of chloroplasts and treatment of them with aldehydes

Intact chloroplasts were prepared from field-grown *Spinacia oleracea* L. (cv. Akution; Sakata Seed, Yokohama, Japan) leaves (Mano et al. 2001). All procedure was done at 4°C. After purified by Percoll (GE Biomedicals, Tokyo, Japan) density gradient centrifugation, chloroplasts were suspended in 0.3 M sorbitol, 10 mM NaCl, 1 mM $MgCl_2$, 1 mM ascorbic acid (Asc), 0.5 mM diethylenetriamine-*N*,*N*,*N*′,*N*′′,*N*′′-pentaacetic acid, 0.5 mM sodium pyrophosphate, and 50 mM Hepes-NaOH, pH 7.6 (chloroplast medium). Chloroplast suspension (2 mg chlorophyll (Chl) ml^{-1}) was incubated in 2 mM aldehyde in darkness at 25°C for 8 min, diluted with 9 volumes of chloroplast medium, and chilled on ice until assays.

Assays

Chloroplasts at 30 μg Chl ml^{-1} in chloroplast medium, supplemented with 10 mM $NaHCO_3$, 0.05 mM Na-phosphate and 0.5 mM GAP, were illuminated at 2,000 μmol photons m^{-2} s^{-1} with white light from a tungsten lamp at 25°C, and the O_2 evolution rate was monitored with a Clark-type O_2 electrode (Hansatech, King's Lynn, UK). GAP was added to start the CO_2-fixation smoothly. For CO_2-fixation assay, Na [^{14}C]-HCO_3 at 10 mM was used in the above medium. The reaction was stopped by adding 37 volumes of ethanol. After removing untreated CO_2 by acidifying the medium with glacial acetic acid, incorporation of radioactivity into organic acids was determined. For the photoreduction of 3-phosphoglyceric acid (PGA), chloroplasts at 30 μg Chl ml^{-1} in chloroplast medium, supplemented with 0.05 mM Na-phosphate and 1 mM PGA, were illuminated and the O_2 evolution was determined as described above. For the electron transport assay, 30 μg Chl aliquots of the chloroplasts ruptured in 5 mM Hepes-NaOH, pH 7.6, were transferred to the reaction medium containing 50 mM Tricine-KOH, pH 7.5, 20 mM NaCl, 0.5 μM nigericin, 0.5 mM $NADP^+$ and 10 μM ferredoxin (Fd) and the O_2 evolution under illumination was determined as described above. Aldolase activity was determined with an enzyme-coupled assay (Haake et al. 1998). Assay media for fructose-1,6-bisphosphatase (FBPase; Charles and Halliwell 1980) and sedoheptulose-1,7-bisphosphatase (SBPase; Harrison et al. 1998) contained DTT at 1.0 and 7.2 mM, respectively, for fully reducing the redox-regulated thiols. Enzymes were pretreated in the assay medium for 10 min, and then activities were determined in the same medium. Similarly, assay media for glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Mano et al. 2001) and phosphoribulokinase (PRK; Porter et al. 1986) contained the reduced form of glutathione (GSH) at 10 mM. Enzymes were pretreated for 10 min. Rubisco was activated with Mg^{2+} and CO_2 prior to the assay, and the incorporation of [^{14}C]-CO_2 to PGA was determined (Shen and Ogren 1992). Total glutathione [GSH + oxidized form of glutathione (GSSG)] was determined as follows: Chloroplast suspension was mixed with four volumes of 0.5% sulfosalicylic acid. After centrifugation, the collected supernatant was mixed with nine volumes of 100 mM Hepes-KOH, pH 7.4, containing 0.5 mM EDTA. The content of glutathione was determined by a cycling assay using glutathione reductase and 5,5′-dithiobis-2-nitrobenzoic acid (Roberts and Francetic 1993).

Results

Comparison of the toxicity of aldehydes

Toxicity of aldehydes was evaluated as an inhibition of the CO_2-supported electron transport activity as determined by O_2 evolution (designated 'CO_2-photoreduction') in chloroplasts. Intact chloroplasts isolated from spinach leaves showed a CO_2-photoreduction activity at 40–80 μmol O_2 mg Chl^{-1} h^{-1}. This activity was inhibited in a time- and concentration-dependent manner by 2-propenal (acrolein), HNE, and (*E*)-2-butenal (crotonaldehyde), the most reactive aldehydes known (Esterbauer et al. 1991) (Fig. 1). The lowered activity was not recovered by washing the chloroplasts with chloroplast medium, indicating irreversible inactivation. The envelope intactness as determined by the ferricyanide method (Heber and Santarius 1970) was unaffected by acrolein or HNE (97% intactness, before and after the treatment), indicating that the loss of CO_2-photoreducing activity was not due to the disintegration of envelopes. Because the CO_2-photoreduction here was determined as O_2 evolution, it was possible that the evaluation of inactivation was affected by changes, if any, in the O_2 budget. For example, if Asc peroxidase was inactivated, H_2O_2 would accumulate, leading to an increase in the O_2 consumption (Mano et al. 2001). In such a case, O_2 evolution due to CO_2 fixation could be partially masked by the O_2 consumption, leading to an overestimation of the inactivation. We confirmed that the [^{14}C]-CO_2 incorporation in chloroplasts was inactivated by acrolein in a concentration dependency similar to that for the O_2 measurement (data

not shown). In addition, Asc peroxidase was insensitive to acrolein (J. Mano, Science Research Center, and H. Mizoguchi, Graduate School of Agriculture, both Yamaguchi University, unpublished result). Thus, the decrease in the O_2 evolution observed in Fig. 1 represented that of CO_2-fixation.

We selected 17 species of aldehydes (carbon chain length 1–9) that have been known to occur in living cells and evaluated their toxicity to photosynthesis (Table 1). Their occurrence in plants is summarized in the Table legend. Ten of them were the aldehydes of saturated carbon chain, ranging from C_1 to C_9, some containing additional hydroxy- or oxo-groups. Glycolaldehyde and glyceraldehyde, by-products of sugar metabolisms, were added to the list because they are inhibitors of PRK (Miller and Canvin 1989), although their occurrence in plants is uncertain. Seven of them were unsaturated aldehydes of C_3–C_9. Most of the tested aldehydes inactivated the CO_2-photoreduction significantly, except for glycolaldehyde, glyceraldehyde, MDA and butyraldehyde. The toxicity of tested aldehydes was attributable to their aldehyde moiety because their corresponding alcohols (methanol, ethanol, propanol, 2-propenol, butanol, 2-butenol, *n*-hexanol, (*E*)-2-hexenol, *n*-nonanol, and (*E*)-2-nonenol) or carboxylic acids (formic acid, acetic acid, acrylic acid, propionic acid, butyric acid, (*Z*)-3-hexenoic acid, hexanoic acid, and nonanoic acid) showed no toxicity at 2 mM (data not shown). As expected, toxicity of the 2-alkenals acrolein, crotonaldehyde, (*E*)-2-hexenal and (*E*)-2-nonenal was significantly higher than that of their corresponding *n*-alkanals, i.e. propionaldehyde, butyraldehyde, *n*-hexanal and *n*-nonanal, respectively. This explains the stress-defensive effects of AER overexpressed in tobaccos (Mano et al. 2005), which converts (*E*)-2-alkenals to *n*-alkanals (Mano et al. 2002). There was no obvious correlation between the toxicity and the carbon chain length; acrolein was the strongest, followed by HNE, (*E*)-2-hexenal and HHE. Among the tested 2-alkenals, crotonaldehyde and (*E*)-2-nonenal were the weakest, causing 29% inactivation.

As for C_6-unsaturated aldehydes, (*Z*)-3-hexenal was as toxic as (*E*)-2-hexenal and more than HHE. This order of toxicity in these aldehydes was unpredictable from their chemical reactivity, and suggests a rapid metabolism of C_6-aldehydes in the chloroplast (see "Discussion").

MDA is contained in *A. thaliana* leaves at ca. 3 nmol (g fresh weight)$^{-1}$ (Weber et al. 2004). Methylglyoxal occurs at 50–70 μM in non-stressed leaves and roots of several species of plants, and is raised up to 200 μM upon NaCl stress (Yadav et al. 2005), to cause cellular injury (Singla-Pareek et al. 2003). Glyceraldehyde and glycolaldehyde inhibit PRK (Miller and Canvin 1989). These oxygenated aldehydes had relatively weak toxicity (5–17% inactivation) compared with 2-alkenals. In all tested aldehydes, acrolein showed the strongest inactivation, and HNE the second strongest.

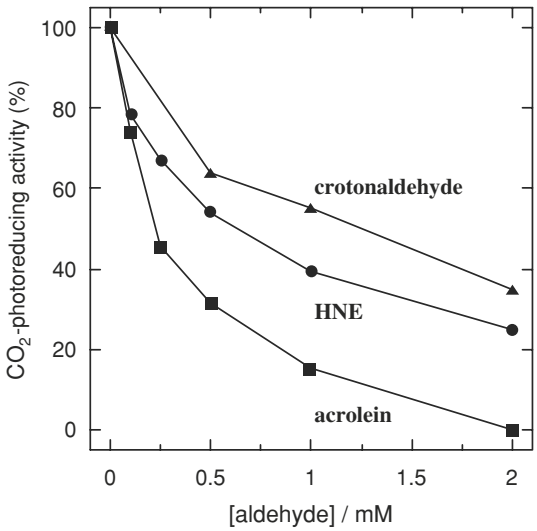


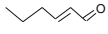
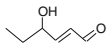
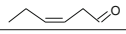
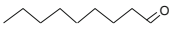
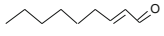
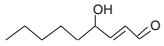
Fig. 1 Concentration dependence of the inactivation of the CO_2-photoreduction in chloroplasts by acrolein, HNE and crotonaldehyde. Chloroplasts (2 mg Chl ml^{-1}) in chloroplast medium were treated with an aldehyde at indicated concentrations in darkness for 8 min at 25°C, diluted with 9 volumes of chloroplast medium, and chilled on ice. CO_2-photoreducing activity was stable after dilution, i.e., the dilution and chilling virtually stopped the action of aldehydes

Calvin cycle is more sensitive to 2-alkenals than electron transport chain

In order to identify the targets of 2-alkenals in chloroplasts, we employed acrolein as a representative compound. Chloroplasts were treated with acrolein at various concentrations and the activities of partial photosynthetic reactions were determined (Fig. 2). After the treatment with 2 mM acrolein, the CO_2-photoreducing activity was totally lost, but the thylakoid electron transport chain (from H_2O to Fd) retained 95% activity. Crotonaldehyde or HNE at 5 mM also resulted in the total inactivation of CO_2-photoreduction with more than 95% electron transport activity retained. Thus, the thylakoid electron transport chain is rather insensitive to 2-alkenals.

The Fd-photoreducing activity was unaffected in the above experiments, but there was a possibility that the endogenous Fd was inactivated, so that the photoproduction of NADPH in chloroplasts was impaired. It was also unclear whether or not photophosphorylation was functioning because in the above experiment electron transport was determined in the presence of an uncoupler. In order to verify the supply of both NADPH and ATP to the Calvin cycle enzymes in the acrolein-treated chloroplasts, we determined the photoreduction of PGA, in which ATP-dependent 3-phosphoglycerate kinase and NADPH-dependent GAPDH are involved (Fig. 4, "$H_2O \rightarrow$ PGA"). The PGA

Table 1 Toxicity of various aldehydes on chloroplast photosynthesis

C-chain length	Inactivation of CO_2-photoreduction (%)					
	saturated (*n*-alkanals)			unsaturated (2-alkenal)[a]		
1	formaldehyde	HCHO	23 ± 1	-		
2	acetaldehyde	CH_3CHO	20 ± 1	-		
	glycolaldehyde		5 ± 3			
3	propionaldehyde		17 ± 4	acrolein		100
	MDA		6 ± 4			
	methylglyoxal		17 ± 4			
	glyceraldehyde		7 ± 15			
4	butyraldehyde		5 ± 2	crotonaldehyde		29 ± 3
6	*n*-hexanal		23 ± 5	(*E*)-2-hexenal		45 ± 3
				HHE		34 ± 3
				(*Z*)-3-hexenal[a]		48 ± 3
9	*n*-nonanal		11 ± 3	(*E*)-2-nonenal		29 ± 1
				HNE		70 ± 1

[a] (*Z*)-3-Hexenal is not a 2-alkenal.

Chloroplasts (2 mg Chl ml^{-1}) were treated with an aldehyde at 2 mM in darkness at 25°C for 8 min, diluted with 9 volumes of chloroplast medium, and chilled on ice. Activity of the control (incubated without aldehydes) was 45 μmol O_2 mg Chl^{-1} h^{-1}. Average of 3 runs ± standard deviation. The occurrence of tested aldhydes in plants has been reported as follows: Formaldehyde and acetaldehyde as leaf volatile compounds (Fall 1999), propionaldehyde, *n*-hexanal and *n*-nonanal in fruits of various species (Nursten and Williams 1967) and in leaf volatiles (Hatanaka 1993). MDA in *A. thaliana* leaves (Weber et al. 2004) and in chloroplasts of *A. thaliana* and spinach (Yamauchi et al. 2008). Methylglyoxal in leaves and roots of *Pennisetum glaucum*, *Oriza sativa*, *tobacco*, and *Brassica juncea* (Yadav et al. 2005). Acrolein in strawberry and raspberry fruits (Nursten and Williams 1967) and in volatiles emitted from the leaves of *Alchornea sidifolia*, *Cecropia pachystachia*, *Syagrus romanzoffiana* and *Ficus benjamina* (Carvalho et al. 2005). Crotonaldehyde in strawberry fruits (Nursten and Williams 1967) and in leaf volatiles from *Ficus benjamina* (Carvalho et al. 2005). (*E*)-2-Hexenal in volatiles from leaves of many species (Hatanaka 1993). HHE in barley leaf extract (Kohlmann et al. 1999). (*E*)-2-Nonenal in volatiles from tomato leaves (Wang et al. 2001). HNE in *Phaseolus vulgaris* leaves (Muckenschnabel et al. 2001). (*Z*)-3-Hexenal is a leaf volatile enzymatically formed from 13-hydroperoxide of linolenic acid (Matsui 2006), and is a precursor of (*E*)-2-hexenal and HHE (Weichert et al. 2000)

photoreduction was inactivated by 40%, but not totally, by 2 mM acrolein. This partial loss of the PGA-photoreduction can be explained by a partial inactivation of GAPDH (described below). The activity remaining at a 60% level indicated that both $NADP^+$-photoreduction and photophosphorylation were active, at least partially.

Saturated aliphatic aldehydes such as propionaldehyde and *n*-nonanal also caused loss of the PGA photoreduction by 21 and 13%, respectively, but glycolaldehyde and glyceraldehyde did not (Miller and Canvin 1989). These results indicate that the Calvin cycle is more sensitive than the thylakoid electron transport chain to the LOOH-derived aldehydes.

2-Alkenals inactivate multiple enzymes in the Calvin cycle

It was highly probable that acrolein targeted the thioredoxin-regulated enzymes such as FBPase and SBPase. 2-Alkenals electrophilically attack the thiol group and readily form a Michael adduct (Esterbauer et al. 1991). Acrolein could modify the redox-regulated cysteines (Cys) in these enzymes when they were in the reduced state. Another possibility was that 2-alkenals reacted with the imidazole group in histidine and the ε-amino group of lysine (Lys) (Uchida 2005), causing the loss of function of the target proteins. Therefore multiple enzymes in the Calvin cycle are candidates for covalent modification by acrolein. In order to evaluate the acrolein-mediated inactivation of the Calvin cycle enzymes, we extracted stroma fraction from acrolein-treated chloroplasts and determined their activities (Fig. 3).

Activities of the thioredoxin-regulated enzymes PRK, GAPDH, SBPase, and FBPase were determined under reducing conditions made by GSH or DTT (see "Materials and methods"). The treatment with 2 mM acrolein decreased the activities of these enzymes by 90, 73, 63 and 20%, respectively, of the untreated controls, even under the reduced condition. This indicated that acrolein modified

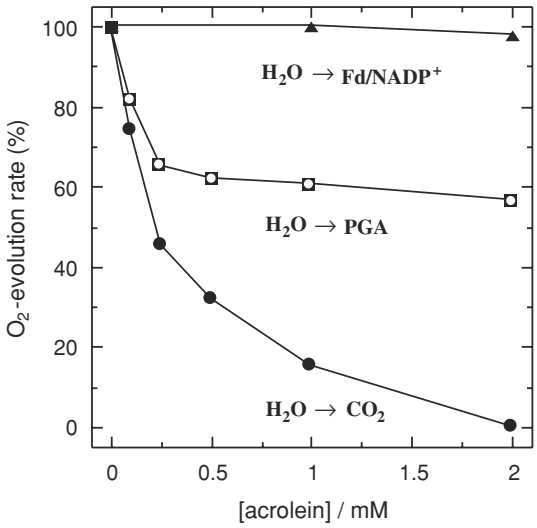


Fig. 2 Effects of acrolein on partial reactions of photosynthesis in the chloroplast. Chloroplasts were treated with acrolein at indicated concentrations as in Fig. 1. Assays were completed within 2 h after treatment. An aliquot of 30 µg of Chl was used for each assay, as determined by the O_2-evolution, as described in "Materials and methods". Average of 3 runs. Control rates of O_2 evolution supported by distinct acceptors (in µmol (mg Chl)$^{-1}$ h^{-1}) were as follows: CO_2, 40 ± 3; PGA, 105 ± 7; $NADP^+$, 145 ± 11

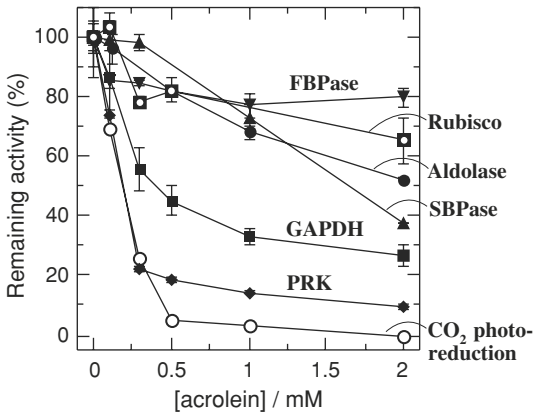


Fig. 3 Inactivation of the Calvin-cycle enzymes by the acrolein treatment of chloroplasts. Chloroplasts were treated with acrolein, and the CO_2-photoreduction was assayed as in Fig. 1. For enzyme assays, treated chloroplasts were ruptured by dilution with 9 volumes of medium for the subsequent enzyme assay and centrifuged at 6,500×*g* for 1 min; the resulting supernatant was then collected. Enzyme activities were determined as described in "Materials and methods". Activities of 100%, corrected for the Chl content of the original chloroplasts (average and standard deviation of 3 runs), were as follows: aldolase, 280 ± 27 µmol mg Chl^{-1} h^{-1}; FBPase, 238 ± 14 µmol mg Chl^{-1} h^{-1}; GAPDH, 640 ± 88 µmol NADPH mg Chl^{-1} h^{-1}; PRK, 397 ± 9 µmol NADPH mg Chl^{-1} h^{-1}; Rubisco, 57.7 ± 2.8 µmol CO_2 mg Chl^{-1} h^{-1}; and SBPase, 67.4 ± 0.4 µmol phosphate mg Chl^{-1} h^{-1}. The CO_2 photoreduction rate before treatment was 63 µmol mg Chl^{-1} h^{-1} (average of two runs)

these enzymes to the inactive forms that were not recovered by reduction. Aldolase and Rubisco were also inactivated by 48 and 35%, respectively. Thus a wide range of enzymes in the Calvin cycle was affected by acrolein, most probably via covalent modification of Cys, Lys and His residues although detailed inactivation mechanisms for these enzymes have yet to be investigated. Other thioredoxin-regulated enzymes such as Rubisco activase can also be targets, and their inactivation by acrolein could contribute to the loss of CO_2-photoreduction.

Glutathione prevents the acrolein toxicity

The above results clearly demonstrated that photosynthesis in chloroplasts is potentially susceptible to aldehydes. We then examined the possibility that GSH protected photosynthesis against acrolein because it has been reported that, in human plasma, GSH prevented the protein modification by 2-alkenals (O'Neill et al. 1994). When chloroplasts were treated with acrolein, the glutathione pool was decreased faster than was photosynthesis inactivated (Fig. 4). This was probably due to that GSH formed the Michael adduct with acrolein faster than were target proteins inactivated. Indeed, supplementation of GSH to the chloroplast suspension suppressed the acrolein-induced inactivation of photosynthesis (Fig. 5). We expected that a gluathione-*S*-transferase might mediate the scavenging of acrolein, but no enzyme activity to catalyze the glutathione-dependent scavenging of acrolein or HNE was detectable in spinach leaves (data not shown). Scavenging of acrolein by GSH

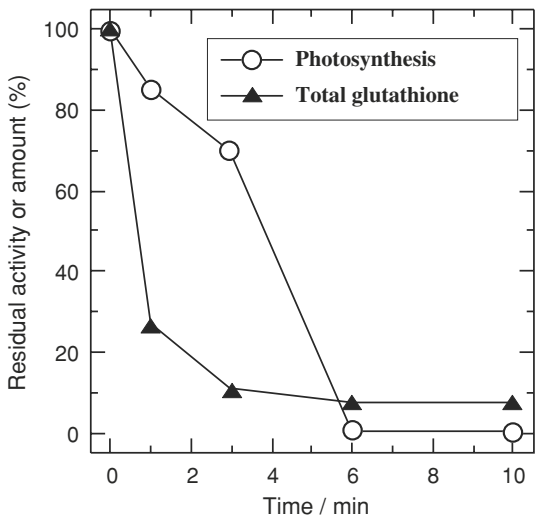


Fig. 4 Acrolein decreases glutathione in chloroplasts. Chloroplasts were treated with 0.5 mM acrolein as in Fig. 1. At the indicated time points, 40 µl aliquots were sampled and mixed with 200 µl sulfosalicylic acid solution [5% (v/v)], for determination of total glutathione (see "Materials and methods"). In a separate incubation, CO_2 photoreduction in treated chloroplasts was assayed as in Fig. 1. Control values for 100% were 57.1 µmol CO_2 mg Chl^{-1} h^{-1} and 81.6 nmol glutathione (GSH + 2 × GSSG) mg Chl^{-1}, which corresponded to the stromal concentration of 3.5 mM (stroma volume 23.0 µl mg Chl^{-1}; Heldt et al. 1973), 90% of which in the reduced form (average of two runs)

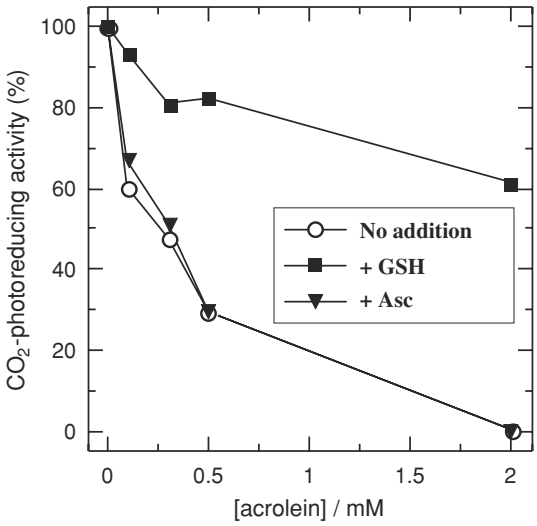


Fig. 5 Effects of GSH and Asc on the acrolein-induced inhibition of photosynthesis. Chloroplasts were incubated in 10 mM GSH or 10 mM Asc in chloroplast medium for 2 min at 25°C, then acrolein was added to the medium, to give the indicated concentration. After 8 min incubation, CO_2 photoreduction was determined as in Fig. 1. Control value for 100% was 50.6 µmol CO_2 mg Chl^{-1} h^{-1}. The CO_2-photoreduction activity was not affected by 10 min-incubation in 10 mM GSH or Asc (average of two runs)

was most probably due to its chemical action because another thiol compoud DTT at 10 mM also reduced the acrolein toxicity to photosynthesis i.e., 60% activity was retained after 2 mM acrolein treatment. In contrast, Asc at 10 mM did not show any protective effect (Fig. 5). These results indicate that GSH, but not Asc, provides as the primary defense against 2-alkenals in plant cells.

Discussion

2-Alkenals are more toxic to photosynthesis than other types of stress-related aldehydes

The present results show that Calvin cycle enzymes are potential targets of LOOH-derived 2-alkenals, as are mitochondrial respiration enzymes (Taylor et al. 2002, Winger et al. 2005). When the aldehydes of the same carbon chain length were compared, 2-alkenals showed higher toxicity than *n*-alkanals for C_3-, C_4-, C_6- and C_9-aldehydes. These results can explain our previous observation that AER protected the transgenic tobaccos against photooxidative stress (Mano et al. 2005) if acrolein, crotonaldehyde, (*E*)-2-hexenal, HHE, (*E*)-2-nonenal or HNE was increased by the stress treatments.

C_6-aldehydes are typical 'chloroplast aldehydes', and hence the effects of their individual species are quite important. One unexpected and interesting result was that HHE showed lower toxicity than (*E*)-2-hexenal (Table 1) in spite that the former has a higher electrophilicity due to the hydroxyl group at C4-position (Esterbauer et al. 1991). We infer that HHE might be scavenged in chloroplasts; there should be a scavenging mechanism specific to HHE because this highly reactive aldehyde is constitutively formed in chloroplast as an oxidized product of (*Z*)-3-hexenal (Kohlmann et al. 1999). Another unexpected result was that (*Z*)-3-hexenal showed a similar strength of toxicity to (*E*)-2-hexenal (Table 1) in spite that the former is obviously less electrophilic, because of the lack of conjugated double bonds, than the latter. One explanation is that (*Z*)-3-hexenal might be very rapidly converted to more reactive compounds such as 4-peroxy-(*E*)-2-hexenal by a peroxygenase (Kohlmann et al. 1999) or (*E*)-2-hexenal by the enzyme 3*Z*:2*E*-enal isomerase (Noordermeer et al. 1999), although their occurrence in chloroplasts has not been verified. Further investigations into the metabolism of C_6 aldehydes and the action of (*Z*)-3-hexenal on photosynthetic reactions are required.

MDA has been recognized to be relevant to environmental stress in plants (see "Introduction"). As compared with 2-alkenals such as HNE, however, it showed only weak toxicity to chloroplasts. This is not surprising when one considers that the reactivity of MDA is much lower than that of HNE (Esterbauer et al. 1991). MDA can be as harmful as 2-alkenals when the tissue content of the former becomes 10 to 20-fold higher than those of the latter, as in the infected *Phaseolus vulgaris* leaves (Muckenschnabel et al. 2001). Another oxygenated C_3 aldehyde methylglyoxal is obviously a major toxin in NaCl stress because glyoxalases improved the stress tolerance (Singla-Pareek et al. 2003). To chloroplast photosynthesis, however, methylgyoxal showed moderate toxicity, in comparison with 2-alkenals (Table 1). Probably the target sites of methylgyoxal in NaCl stress are different from chloroplast photosynthesis reactions.

Consequences of the toxicity of 2-alkenals

Current results with acrolein (Figs. 2–5) can be provided as models showing the consequences of enhanced 2-alkenal levels in leaves. The present data show that GSH is very likely more important than Asc in protecting chloroplast processes against acrolein. First, chloroplast GSH contents were rapidly decreased by acrolein (Fig. 4). Second, pre-addition of GSH but not Asc to chloroplast suspension decreased the sensitivity of photosynthesis to acrolein (Fig. 5). Although we cannot exclude that some of the protective effect of GSH may have been caused by a direct interaction with acrolein in the medium, the two observations together suggest that chloroplast GSH concentration is an important factor determining conjugation rate and protection of photosynthesis against this reactive aldehyde.

This scavenging reaction through the formation of Michael adducts (conjugates), unlike the oxidation of GSH to GSSG, immediately leads to a decrease in the total glutathione pool (GSH + GSSG) (Fig. 4). Then GSH in chloroplasts should be supplemented by de novo synthesis within the plastid and by import from the cytosol. When the consumption exceeds the supplementation, the glutathione pool will be decreased. Two effects are expected. One is the inactivation of target enzymes by the enhanced 2-alkenals (Fig. 3; mechanism discussed below). The other is a loss of activity control through glutathionylation of aldolase, triose-phosphate isomerase, thioredoxin f, and GAPDH (Ito et al. 2003; Michelet et al. 2005; Zaffagnini et al. 2007).

The effects of acrolein on FBPase and PRK are interpreted in a common mechanism. These enzymes exhibited an acute inactivation phase below 0.5 mM acrolein and a relatively stable phase in its higher concentration (Fig. 3). The following mechanism explains such a biphasic inactivation: Acrolein reacts primarily with the redox-regulated thiols on the enzyme and converts it to an inactive form. The disulfide form of the enzyme is much less sensitive to acrolein (Fig. 6). When acrolein was added to chloroplasts, it should cause two effects on these thiol-regulated enzymes; the acceleration of the thiol oxidation to disulfide by consuming stromal GSH, and the modification of thiols. On assay of the resulting population of the enzyme, the disulfide form was reactivated by reduction, while the acrolein-modified forms did not restore the activity even by reduction. Higher sensitivity of PRK than that of FBPase (Fig. 3) can be explained by the difference of the ratio of these two forms, which could be ascribed to the difference of the midpoint potential of the thiols, as follows. The midpoint potential of the thiols are ca. –315 mV in PRK and –350 mV in FBPase (Hutchison et al. 2000). Therefore, when the stromal redox status becomes more oxidized due to the consumption of GSH, the former enzyme will stay in the reduced form longer than the latter. This would make PRK more susceptible to the Michael addition of acrolein.

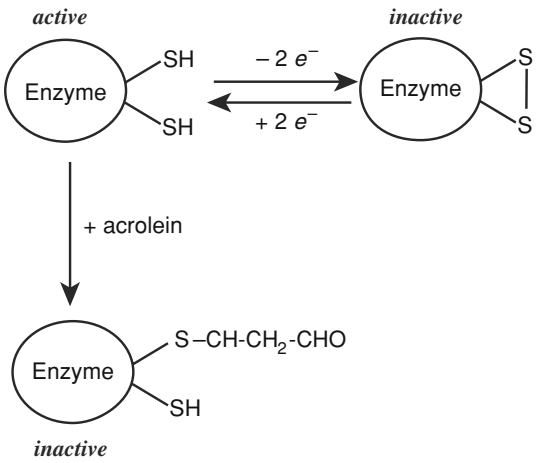


Fig. 6 Modification of a Cys residue(s) on a redox-regulated enzyme with acrolein

Other tested enzymes did not show apparent biphasic inactivation although some of them also have redox-regulated thiols. The inactivation of these enzymes might be caused by the modification of not only the redox-regulated thiols but also other amino acids such as Lys and His, as observed for the inactivation of rabbit muscle GAPDH by HNE (Ishii et al. 2003). Thus, Calvin cycle enzymes are inactivated by acrolein in various modes, to different extents. We are investigating the inactivation mechanisms of several enzymes with various aldehydes. The above-mentioned mechanisms of acrolein's action on photosynthesis can be basically applied to other 2-alkenals, although each aldehyde will show different strength of effect, as in Fig. 1, and there may be specific effects in certain combinations of aldehydes and targets.

In severe oxidative stress, not only the Calvin cycle but also mitochondrial respirations will be inactivated by 2-alkenals (Taylor et al. 2002; Winger et al. 2005). This will lead to the loss of energy-consumption capacity in the cell and exacerbate light-excess status and increase the production of reactive oxygen species. Scavenging of 2-alkenals would be thus critical to protect the target enzymes from inactivation at early stages of stress, and to prevent further development of oxidative injury, by preserving the electron sink capacity in leaf cells.

Physiological relevance

In what physiological situations the above-mentioned toxicity of 2-alkenals can be significant? As described in Table 1 legend, plant tissues can generate various 2-alkenals. Kohlmann et al. (1999) determined the HHE content in barley leaves as high as 17 nmol (g fresh weight)$^{-1}$. This value corresponds to μM levels in cells when homogeneous distribution of the aldehyde in the tissue is assumed, and can be sub-mM level when its compartmentation in the cell is considered. Even higher aldehyde contents are expected under oxidative stress conditions. The HNE content in *Phaseolus vulgaris* leaves was increased 100-fold or more, during the oxidative stress induced by bacterial infection (Muckenschnabel et al. 2001). Similarly, in photooxidative status induced by various environmental stresses, 2-alkenal levels will be increased. Indeed, the emission of (*E*)-2-hexenal from leaves was increased by heat stress or strong light illumination to *Phragmites australis* plants, and by photoinhibition treatment of the *A. thaliana* NPQ1 mutant (Loreto et al. 2006). For evaluation of intracellular aldehyde levels we have recently developed an analysis method (Matsui et al. 2009) and obtained preliminary results that

thylakoid membranes contained crotonaldehyde, (*E*)-2-pentenal and HHE, at sub-mM to mM on the thylakoid volume, in non-stressed preparations (S. Khorobrykh, J. Mano, Y. Iijima and D. Shibata (both Kazua DNA Institute, Kisaradzu, Japan), unpublished data), and that they were increased several fold by strong illumination of the leaves (J. Mano, S. Khorobrykh, K. Tokushige (Graduate School of Agriculture, Yamaguchi University), Y. Iijima and D. Shibata, unpublished data). Thus 2-alkenals are endogenously produced in chloroplasts and their levels can be increased to toxic levels by environmental stresses. In order to evaluate the in vivo toxicity of 2-alkenals, comprehensive analysis of the produced species and their levels will be necessary.

Acknowledgments This work was supported by Yamada Science Foundation and by Japanese Society for the Promotion of Science Grant-in-Aid for the Promotion of Science (No. 15570039) to J.M. The authors are grateful to Profs. S. Shigeoka (Faculty of Agriculture, Kinki University) and K. Matsui (Graduate School of Medicine, Yamaguchi University) and Dr. S. Khorobrykh (Science Research Center, Yamaguchi University) for useful discussions, Ms. M. Onaga for technical advice, Y. Morita, H. Kumura, M. Nagata, K. Hatanaka and T. Hirota for their technical assistance, and the reviewers for helpful suggestions.

References

Alméras E, Stolz S, Vollenweider S, Reymond P, Mène-Saffrané L, Farmer EE (2003) Reactive electrophile species activate defense gene expression in *Arabidopsis*. Plant J 34:205–216

Arlt S, Beisiegel U, Kontush A (2002) Lipid peroxidation in neurodegeneration: new insights into Alzheimer's disease. Curr Opin Lipidol 13:289–294

Asada K (2006) Production and scavenging of reactive oxygen species in chloroplasts and their functions. Plant Physiol 141:391–396

Blée E (1998) Phytooxylipins and plant defense reactions. Prog Lipid Res 37:33–72

Burcham PC (1998) Genotoxic lipid peroxidation products: their DNA damaging properties and role in formation of endogenous DNA adducts. Mutagenesis 13:287–305

Carvalho LRF, Vasconcellos PC, Mantovani W, Pool CS, Pisani SO (2005) Measurements of biogenic hydrocarbons and carbonyl compounds emitted by trees from temperate warm Atlantic rainforest, Brazil. J Environ Monit 7:493–499

Charles SA, Halliwell B (1980) Properties of freshly purified and thiol-treated spinach chloroplast fructose bisphosphatase. Biochem J 185:689–693

Chen J, Schenker S, Frosto TA, Henderson GI (1998) Inhibition of cytochrome *c* oxidase activity by 4-hydroxynonenal (HNE). Role of HNE adduct formation with the enzyme subunits. Biochim Biophys Acta 1380:336–344

Comporti M (1985) Biology of disease. Lipid peroxidation and cellular damage in toxic liver injury. Lab Invest 53:599–623

Esterbauer H, Schaur R, Zollner JH (1991) Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Rad Biol Med 11:81–128

Fall R (1999) Biogenic emissions of volatile organic compounds from higher plants. In: Hewitt CN (ed) Reactive hydrocarbons in the atmosphere. Academic Press, San Diego, pp 41–96

Farmaki T, Sanmartín M, Jiménez P, Paneque M, Saz C, Vancanneyt G, León J, Sánchez-Serrano JJ (2007) Differential distribution of the lipoxygenase pathway enzymes within potato chloroplasts. J Exp Bot 58:558–568

Haake V, Zrenner R, Sonnewald U, Stitt M (1998) A moderate decrease of plastid aldolase activity inhibits photosynthesis, alters the levels of sugars and starch, and inhibits growth of potato plants. Plant J 14:147–157

Harrison EP, Willingham NM, Lloyd JC, Raines CA (1998) Reduced sedoheptulose-1,7-bisphosphatase levels on transgenic plants lead to decreased photosynthetic capacity and altered carbohydrate accumulation. Planta 204:27–36

Hatanaka A (1993) The biogeneration of green odour by green leaves. Phytochemistry 34:1201–1218

Heber U, Santarius KA (1970) Direct and indirect transfer of ATP and ADP across the chloroplast envelope. Z Naturforsch 25b:718–728

Heldt HW, Werden K, Milovancev M, Geller G (1973) Alkalization of the chloroplast stroma caused by light-dependent proton flux into the thylakoid space. Biochim Biophys Acta 314:224–241

Hideg É, Nagy T, Oberschall A, Dudits D, Vass I (2003) Detoxification function of aldose/aldehyde reductase during drought and ultraviolet-B (280–320 nm) stresses. Plant Cell Environ 26:513–522

Hodgson RAJ, Raison JK (1991) Lipid peroxidation and superoxide dismutase activity in relation to photoinhibition induced by chilling in moderate light. Planta 185:215–219

Humphries KM, Szweda LI (1998) Selective inactivation of α-ketoglutarate dehydrogenase and pyruvate dehydrogenase: Reaction of lipoic acid with 4-hydroxy-2-nonenal. Biochemistry 37:15835–15841

Hutchison RS, Groom Q, Ort DR (2000) Differential effects of chilling-induced photooxidation on the redox regulation of photosynthetic enzymes. Biochemistry 37:6679–6688

Ishii T, Tatsuda E, Kumazawa S, Nakayama T, Uchida K (2003) Molecular basis of enzyme inactivation by an endogenous electrophile 4-hydroxy-2-nonenal: Identification of modification sites in glyceraldehyde-3-phosphate dehydrogenase. Biochemistry 42:3474–3480

Ito H, Iwabuchi M, Ogawa K (2003) The sugar-metabolic enzymes aldolase and triose-phosphate isomerase are targets of glutathionylation in *Arabidopsis thaliana*: detection using biiotinylated glutathione. Plant Cell Physiol 44:655–660

Kohlmann M, Bachmann A, Weichert H, Kolbe A, Balkenhohl T, Wasternack C, Feussner I (1999) Formation of lipoxygenase-pathway-derived aldehydes in barley leaves upon methyl jasmonate treatment. Eur J Biochem 260:885–895

Kotchoni SO, Kuhns C, Ditzer A, Kirch H-H, Bartels D (2006) Overexpression of different *aldehyde dehydrogenase* genes in *Arabidopsis thaliana* confers tolerance to abiotic stress and protects plants against lipid peroxidation and oxidative stress. Plant Cell Environ 29:1033–1048

Loreto F, Barta C, Brilli F, Nogues I (2006) On the induction of volatile organic compound emissions by plants as consequence of wounding or fluctuations of light and temperature. Plant Cell Environ 29:1820–1828

Mano J, Ohno C, Asada K (2001) Chloroplastic ascorbate peroxidase is the primary target of methylviologen-induced photooxidative stress in spinach leaves: its relevance to monodehydroascorbate radical detected with in vivo ESR. Biochim Biophys Acta 1504:275–287

Mano J, Torii Y, Hayashi S, Takimoto K, Matsui K, Nakamura K, Inzé D, Babiychuk E, Kushnir S, Asada K (2002) The NADPH:quinone oxidoreductase P1-ζ-crystallin in *Arabidopsis* catalyzes the α, β-hydrogenation of 2-alkenals: detoxication of the lipid peroxide-derived reactive aldehydes. Plant Cell Physiol 23:1445–1455

Mano J, Belles-Boix E, Babiychuk E, Inzé D, Torii Y, Hiraoka E, Takimoto K, Asada K, Slooten L, Kushnir S (2005) Protection

against photooxidative injury of tobacco leaves by 2-alkenal reductase. Detoxication of lipid peroxide-derived reactive carbonyls. Plant Physiol 139:1773–1783

Matsui K (2006) Green leaf volatiles: hydroperoxide lyase pathway of oxylipin metabolism. Curr Opin Plant Biol 9:274–280

Matsui K, Sugimoto K, Kakumyan P, Khorobrykh SA, Mano J (2009) Volatile oxylipins formed under stress in plants. In: Armstrong D (ed) Lipidomics: technology and applications. Humana Press, Totowa

Michelet L, Zaffagnini M, Varchand C, Collin V, Decottingnies P, Tsan P, Lancelin J-M, Trost P, Miginiac-Maslow M, Noctor G, Lamaire SD (2005) Glutathionylation of chloroplast thioredoxin f is a redox signaling mechanism in plants. Proc Natl Acad Sci USA 102:16478–16483

Millar AH, Leaver CJ (2000) The cytotoxic lipid peroxidation product, 4-hydroxy-2-nonenal, specifically inhibits decarboxylating dehydrogenases in the matrix of plant mitochondria. FEBS Lett 481:117–121

Miller AG, Canvin DT (1989) Glycoladehyde inhibits CO_2 fixation in the cyanobacterium *Synechococcus* UTEX 625 without inhibiting the accumulation of inorganic carbon or the associated quenching of chlorophyll *a* fluorescence. Plant Physiol 91:1044–1049

Mishra R, Singhal GS (1992) Function of photosynthetic apparatus of intact wheat leaves under high light and heat stress and its relationship with peroxidation of thylakoid lipids. Plant Physiol 98:1–6

Moran JF, Becana M, Iturbe-Ormaetxe I, Frechilla S, Klucas RV, Aparicio-Tejo P (1994) Drought induces oxidative stress in pea plants. Planta 194:346–352

Muckenschnabel I, Williamson B, Goodman BA, Lyon GD, Stewart D, Deighton N (2001) Markers for oxidative stress associated with soft rots in French beans (*Phaseolus vulgaris*) infected by *Botrytis cinerea*. Planta 212:376–381

Nair U, Bartsch H, Nair J (2007) Lipid peroxidation-induced DNA damage in cancer -prone inflammatory diseases: a review of published adduct types and levels in humans. Free Rad Biol Med 43:1109–1120

Noordermeer MA, Veldink GA, Vliegenthart JFG (1999) Alfalfa contains substantial 9-hydroperoxide lyase activity and a 3*Z*:2*E*-enal isomerase. FEBS Lett 443:201–204

Nursten HE, Williams AA (1967) Fruit aromas: a survey of compounds identified. Chem Ind 1967:486–497

O'Brien P, Siraki AG, Shangari N (2005) Aldehyde sources, metabolism, molecular toxicity mechanisms, and possible effects on human health. Crit Rev Toxicol 35:609–662

O'Neill CA, Halliwell B, van der Vliet A, Davis PA, Packer L, Trischler H, Strohman WJ, Rieland T, Cross CE, Reznick AZ (1994) Aldehyde-induced protein modifications in human plasma: protection by glutathione and dihydrolipoic acid. J Lab Clin Med 124:359–370

Oberschall A, Deák M, Török K, Sass L, Vass I, Kovács I, Fehér A, Dudits D, Horváth GV (2000) A novel aldose/aldehyde reductase protects transgenic plants against lipid peroxidation under chemical and drought stresses. Plant J 24:437–446

Panagopoulos I, Bornman JF, Björn LO (1992) Response of sugar beet plants to ultraviolet-B (280–320 nm) radiation and *Cercospom* leaf spot disease. Plant J 84:140–145

Porter MA, Milanez S, Stringer CD, Hartman FC (1986) Purification and characterization of ribulose-5-phosphate kinase from spinach. Arch Biochem Biophys 245:14–23

Roberts JC, Francetic DJ (1993) The importance of sample preparation and storage in glutathione analysis. Anal Biochem 211:183–187

Sakaki T, Kondo N, Sugahara K (1983) Breakdown of photosynthetic pigments and lipids in spinach leaves with ozone fumigation: role of active oxygens. Physiol Plant 59:28–34

Sharma PK, Singhal GS (1992) The role of thylakoid lipids in the photodamage of photosynthetic activity. Physiol Plant 86:623–629

Shen JB, Ogren WL (1992) Alteration of spinach ribulose-1,5-bisphosphate carboxylase/oxygenase activase activities by site-directed mutagenesis. Plant Physiol 99:1201–1207

Singla-Pareek SL, Reddy MK, Sopory SK (2003) Genetic engineering of the glyoxalase pathway in tobacco leads to enhanced salinity tolerance. Proc Natl Acad Sci USA 100:14672–14677

Sunkar R, Bartels D, Kirch H-H (2003) Overexpression of a stress-inducible aldehyde dehydrogenase from *Arabidopsis thaliana* in transgenic plants improves stress tolerance. Plant J 35:452–464

Tamoi M, Nagaoka M, Shigeoka S (2005) Immunological properties of sedoheptulose-1,7-bisphosphatase from Chlamydomonas sp. W80. Biosci Biotechnol Biochem 69:848–851

Taylor NL, Day DA, Millar AH (2002) Environmental stress causes oxidative damage to plant mitochondria leading to inhibition of glycine decarboxylase. J Biol Chem 277:42662–42668

Uchida K (2005) Protein-bound 4-hydroxy-2-nonenal as a marker of oxidative stress. J Clin Biochem Nutr 36:1–10

Wang C, Zing J, Chin C-K, Ho C-T, Martin CE (2001) Modification of fatty acids changes the flavor volatiles in tomato leaves. Phytochemistry 58:227–232

Weber H, Chételat A, Reymond P, Farmer EE (2004) Selective and powerful stress gene expression in *Arabidopsis* in response to malondialdehyde. Plant J 37:877–888

Weichert H, Kolbe A, Wasternack C, Feussner I (2000) Formation of 4-hydroxy-2-alkenals in barley leaves. Biochem Soc Trans 28:850–851

Winger AM, Millar AH, Day DA (2005) Sensitivity of plant mitochondrial terminal oxidases to the lipid peroxidation product 4-hydroxy-2-nonenal (HNE). Biochem J 387:865–870

Winger AM, Taylor NL, Heazlewood JL, Day DD, Millar AH (2007) The cytotoxic lipid peroxidation product 4-hydroxy-2-nonenal covalently modifies a selective range of proteins linked to respiratory function in plant mitochondria. J Biol Chem 282:37436–37447

Yadav SK, Singla-Pareek SL, Ray M, Reddy MK, Sopory SK (2005) Methylglyoxal levels in plants under salinity stress are dependent on glyoxalase I and glutathione. Biochem Biophys Res Commun 337:61–67

Yamauchi Y, Furutera A, Seki K, Toyoda Y, Tanaka K, Sugimoto Y (2008) Malondialdehyde generated from peroxidized linolenic acid causes protein modification in heat-stressed plants. Plant Physiol Biochem 46:786–793

Zaffagnini M, Michelet L, Marchand C, Sparla F, Decottignies P, Le Maréchal P, Miginiac-Maslow M, Noctor G, Trost P, Lemaire SD (2007) The thioredoxin-independent isoform of chloroplastic glyceraldehyde-3-dehydrogenase is selectively regulated by glutathionylation. FEBS J 274:212–226