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The Function of Homoglutathione and Hydroxymethylglutathione for the Scavenging of Hydrogen Peroxide

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The function of homoglutathione (γGluCysβAla, hGSH) and hydroxymethylglutathione (γGluCysSer, HMGSH) for the photoreduction of H_2O_2 was investigated. Five to ten percents of the total leaf tripeptide were found in the chloroplasts of the hGSH species *Glycine max* and *Phaseolus coccineus* and of *Triticum aestivum*, a species which has HMGSH in addition to GSH. The K_m of bean glutathione reductase (GR) for oxidized hGSH and for GSSG were nearly the same, whereas GSSG was better substrate for wheat and spinach GR. All GR's showed a much lower affinity to oxidized HMGSH than to GSSG. Intact chloroplasts of bean, soybean and wheat showed a photoreduction of H_2O_2 only when they were isolated in the presence of ascorbate. The reduction rate for wheat chloroplasts was the same as for pea and spinach chloroplasts, whereas bean and soybean chloroplasts had a much lower rate at 25–100 μM H_2O_2. The activities of ascorbate peroxidase and monodehydroascorbate reductase in the chloroplasts of all five species were comparable. The chloroplasts of bean and soybean, however, had no or little dehydroascorbate reductase and GR activities, therefore, ascorbate regeneration by hGSH as a reductant will be of limited significance in both the plants under normal conditions.

Key words: Glutathione — Homoglutathione — Scarlett runner bean (*Phaseolus coccineus*) — Scavenging of hydrogen peroxide — Soybean (*Glycine max*) — Wheat (*Triticum aestivum*).

The essential role of glutathione (γGluCysGly, GSH) as a reductant of oxyradicals is based on the oxidation of the SH-group of the tripeptide to the disulfide form (GSSG). A high ratio of GSH to GSSG is maintained by a reduction of GSSG in a reaction of glutathione reductase (GR, EC 1.6.4.2). The function of glutathione as a reductant becomes evident under conditions of oxidative stress such as exposure to O_3, SO_2, drought or frost, where GSH and GR are induced (for review see Alscher 1989, Smith et al. 1989).

In plants the best documented antioxidant function of glutathione and GR is their participation in the H_2O_2 scavenging of the chloroplasts. H_2O_2 is produced as a result of the Mehler reaction and the disproportionation of O_2^- by SOD and is removed by a reaction sequence involving the redox pairs ascorbate-dehydroascorbate and reduced-oxidized glutathione. The first step of this reaction sequence is catalyzed by ascorbate (AsA) peroxidase (EC 1.11.1.11), which effectively reduces H_2O_2 to water. The primary oxidation product in the ascorbate peroxidase reaction is monodehydroascorbate (MDA, also referred to as ascorbate free radical), which is reduced to AsA by MDA reductase (EC 1.6.5.4) at the expense of NADH or NADPH, or which disproportionates non-enzymatically to dehydroascorbate (DHA) and AsA (Hossain et al. 1984). DHA is reduced to AsA by a reaction with GSH, either non-enzymatically at a low rate or, at much higher rates, catalyzed by DHA reductase (EC 1.8.5.1) (Foyer and Halliwell 1977, Nakano and Asada 1981). GSSG is reduced to GSH by GR using NADPH as electron donor. Since NADPH is supplied by the photosynthetic electron transport chain, the reduction of H_2O_2 is ultimately coupled to the evolution of O_2 (Asada and Badger 1984).

All enzymes involved in the H_2O_2 reduction pathway are also found in the cytosol of leaf cells (Gillham and Dodge 1986) and in non-photosynthetic tissues like soybean root nodules (Dalton et al. 1986) and *Ricinus* endosperm (Klapheck et al. 1990). Therefore the ascor-

Abbreviations: AsA, ascorbate; DHA, dehydroascorbate; GR, glutathione reductase; hGSH/hGSSG, reduced/oxidized homoglutathione; HMGSH/HMGSSG, reduced/oxidized hydroxymethylglutathione; MDA, monodehydroascorbate.

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bate/glutathione cycle for scavenging of H_2O_2 may also exist outside the chloroplasts.

In some plants glutathione is partly or completely replaced by other γ-glutamylcysteinyl tripeptides. The family Fabaceae is characterized by the content of homoglutathione (γGluCysβAla, hGSH): hGSH is the only tripeptide present in the leaves of bean and soybean and other plants of the tribus Phaseoleae and occurs additionally to glutathione in most other tribes of the family (Klapheck 1988, Gekeler et al. 1989). Another tripeptide, hydroxymethylglutathione (γGluCysSer, HMGSH), is present in addition to glutathione in appreciable amounts in many species of the family Poaceae, for example, in wheat and rice, where HMGSH represents 30–70% of the total tripeptide thiols (Klapheck et al. 1992).

In the present paper we have investigated the participation of these glutathione-homologues in the scavenging of H_2O_2. GR's from different plant species as well as that from yeast are able to reduce the disulfide forms of hGSH and HMGSH. The activity of the light-dependent scavenging of H_2O_2 in isolated chloroplasts of bean, soybean and wheat as well as the activity of the involved enzymes and the thiol contents have been determined, and compared to the well characterized systems of spinach and pea chloroplasts.

Materials and Methods

Plant growth—Spinach (*Spinacia oleracea* L. cv. Hiverna) was cultivated under natural light in a glasshouse and the leaves from two- to four-months old plants were used. Scarlett runner bean (*Phaseolus coccineus* L. cv. Preisgewinner), soybean (*Glycine max* L. cv. Kalmit), wheat (*Triticum aestivum* L. cv. Star) and pea (*Pisum sativum* L. cv. Kleine Rheinländerin) were raised in a growth cabinet at 20°C and 80% humidity with a 16-h photoperiod and a light intensity of 120 $\mu E\ m^{-2}\ s^{-1}$. The primary leaves of 13–17-day old bean and soybean plants and the leaves of 10-day old wheat and 15-day old pea plants were used in all studies.

Isolation of chloroplasts—Leaves were cut into small pieces and homogenized in a Braun blender equipped with Kannangara blades with an isolation medium containing 0.33 M sorbitol, 3 mM $MgCl_2$, 2 mM EDTA, 50 mM HEPES, 0.2% (w/v) bovine serum albumin and AsA as indicated, adjusted to pH 7.5 with KOH. Fresh weight input per 100 ml isolation medium and mixing duration were: 25 g and three times each for 2 s for spinach and pea, 25 g and 1.5 s for bean, 12.5 g and ten times each for 2 s for wheat, 12.5 g and three times each for 1 s for soybean. The leaves of soybean were infiltrated with the isolation medium under slight vacuum before mixing.

The homogenate was filtered through four layers of cheesecloth and a nylon net (30 μm pore size) and centrifuged at 2,500 × *g* for 45 s. The pellet was carefully resuspended in isolation medium and was centrifuged at 1,000 × *g* for 90 s. The pellet was again resuspended in isolation medium and layered on 40% (v/v) Percoll containing 0.33 M sorbitol, 50 mM Tricine, 0.2% (w/v) bovine serum albumin and AsA as indicated, adjusted to pH 7.5 with KOH. After centrifugation at 2,500 × *g* for 5 min the pellet containing the intact chloroplasts was resuspended in a minimum volume of isolation medium, leading to a chlorophyll content of 0.3–2 mg ml^{-1}.

Determination of O_2 evolution—Reactions were performed at 30°C and at a light intensity of 550 $\mu E\ m^{-2}\ s^{-1}$ at the surface of the cuvette. O_2 was determined polarographically with a Clark disc electrode (Bachhofer, Hansatech, Reutlingen, F.R.G.). Standard reaction mixture contained 6.25 mM KH_2PO_4, 3.75 mM NH_4Cl and all the components of the isolation medium except AsA, because higher amounts of AsA in the mixture interfere with the O_2-determination. Hill reaction with ferricyanide was determined by injection of 10 mM $K_3[Fe(CN)_6]$. Intactness of chloroplasts was determined by the ferricyanide method of Lilley et al. (1975). For the determination of the H_2O_2-dependent O_2-evolution intact chloroplasts (10–30 μg Chl) were preincubated in 0.5 ml standard reaction mixture for 3 min in the light. Then H_2O_2 was injected. The difference of the time-dependent change of the O_2 concentration before and during the first 30 s after the injection of H_2O_2 was determined. The same measurements were done with another aliquot of the chloroplasts in the dark. The activity of the (light plus H_2O_2)-dependent O_2-evolution was calculated by subtraction of the value determined in the dark from that determined in the light.

Determination of enzyme activities—Activities of AsA peroxidase, MDA reductase, DHA reductase and GR were determined as described by Asada (1984), Hossain et al. (1984), Asada (1984) and Bielawski and Joy (1986), respectively. Enzymes were extracted from the leaves by homogenization of 2 g fresh weight with 10 ml extraction buffer (50 mM HEPES) using a mortar and pestle. The homogenate was centrifuged at 40,000 × *g* for 20 min. Enzymes were released from intact chloroplasts by dilution of the chloroplast suspension with 50 mM HEPES and addition of Triton X-100 (0.1% final concentration), followed by centrifugation at 10,000 × *g* for 6 min. Both enzyme solutions were purified by Sephadex G-25 (PD-10) columns (Pharmacia, F.R.G.) and 20 mM HEPES buffer for elution. Extraction and elution buffers contained 1 mM AsA for spinach, pea and wheat and 5 mM AsA for bean and soybean, and were adjusted to pH 7.5 with KOH. The chromatography of the enzyme solutions led to an increase of the GR activity in all species. Activities of the other enzymes were not altered. Omission of AsA in the extraction buffer reduced AsA peroxidase activity by 28–75%.

Enzymatic reduction of disulfides—Substrate affinity

was determined by incubation of GR (0.005 U each for GSSG and hGSSG, 0.1–0.6 U for HMGSSG, 1 U = 1 μmol min^{-1}) with 5–7 different concentrations of the disulfides in the range of 0.0125–0.5 mM (for HMGSSG: 0.05–1.5 mM). Enzyme activity was measured at 30°C by the decrease in absorbance at 334 nm due to NADPH oxidation according to Bielawski and Joy (1986). K_m and V_{max} values were determined by Lineweaver-Burk plot. GR from yeast (type III), spinach (type VI) and wheat germ (type II) were purchased from Sigma Chemical Co. GR from *Phaseolus coccineus* was isolated from the primary leaves of 14-day old plants and purified 17-fold by ammonium sulfate and acetone precipitation according to Bielawski and Joy (1986) giving a specific activity of 0.3 U mg $protein^{-1}$. GR from *Oryza sativa* var. "Strella" was isolated from 20-day old leaves and purified 14-fold by ammonium sulfate precipitation and gel filtration, giving a specific activity of 1.0 U mg $protein^{-1}$. GSSG was purchased from Boehringer, Mannheim. HMGSH was produced by ABIMED Analysen-Technik GmbH (Langenfeld, F.R.G.). hGSH was isolated from the seeds of *P. coccineus* according to Klapheck (1988). For oxidation to the disulfide form, 10 mM HMGSH or hGSH in water was adjusted to pH 8.0 with NH_4OH, and oxygen was passed through the solution after addition of copper sulfate (2 ppm). The concentrations of hGSSG and HMGSSG were re-checked by HPLC analysis, using GSSG as a standard.

Determination of thiol content and chlorophyll—Thiols were determined by HPLC analysis after reduction with dithioerythritol and monobromobimane derivatization according to Klapheck (1988). Leaves were extracted by homogenization with 0.1 M HCl. Chloroplasts were broken by addition of Triton X-100 and HCl (0.1% v/v and 0.1 M, respectively, final concentrations). The recovery was determined by addition of a known amount of GSH-standard to a parallel sample before extraction and proved to be higher than 95% for leaf extracts and higher than 80% for chloroplast suspensions. Chlorophyll was determined according to Arnon (1949).

Results

Thiol content of leaves and chloroplasts—In the wheat leaves HMGSH is present additionally to GSH and represents one fifth of the total tripeptide content (Table 1). A similar distribution is found in the chloroplasts at 18 nmol GSH and 5 nmol HMGSH mg Chl^{-1}. Assuming that the osmotic volume of chloroplasts is 25 μl mg Chl^{-1}, a value found for spinach (Heldt et al. 1973) and pea (McLaren and Barber 1977), and taking the intactness of the chloroplasts given in Table 1 into account, a total tripeptide concentration of 1.1 mM is calculated for the wheat chloroplasts. The legumes bean and soybean contain hGSH instead of GSH in their leaves and also in chloroplasts. The hGSH content of bean chloroplasts is 8 nmol mg Chl^{-1} and results in the low concentration of 0.4 mM. For soybean chloroplasts a hGSH content of 18 nmol mg Chl^{-1} is found and a concentration of 1.1 mM is calculated. The tripeptide concentration of all three species is lower than that of spinach chloroplasts but for wheat and soybean comparable to that of pea chloroplasts (Table 1). Spinach and pea only have GSH in their leaves and its concentration in chloroplasts is 2.1 mM and 1.2 mM, respectively.

Affinity of GR to the three γ-glutamylcysteinyl tripeptides—A prerequisite for the function of hGSH and HMGSH for the scavenging of H_2O_2 is the effective reduction of the corresponding disulfides by GR. Therefore we determined the affinities of the partially purified GR's from

Table 1 Content of glutathione (γGluCysGly), homoglutathione (γGluCysβAla) and hydroxymethylglutathione (γGluCysSer) in the leaf tissue (Leaf) and in the chloroplasts (Chlp) of five plant species

Species		nmol thiol mg Chl^{-1}			Intactness of Chlp (%)
		GSH	hGSH	HMGSH	
Wheat	Leaf	290±49	0	71±11	
	Chlp	18± 4		5± 1	82±2
Bean	Leaf	1	147±16	0	
	Chlp	1	8± 1	0	79±3
Soybean	Leaf	0	196±23	0	
	Chlp	0	19± 7	0	71±3
Spinach	Leaf	313±31	0	0	
	Chlp	49±10	0	0	95±2
Pea	Leaf	221±95	0	0	
	Chlp	27±10	0	0	91±2

Values are the means±SE of three independent experiments. 0, not detectable.

Table 2 K_m-values of GR's from different sources for γ-glutamylcysteinyl tripeptides, determined from Lineweaver-Burk plots

GR from	K_m (μM)		
	GSSG	hGSSG	HMGSSG
Bean	52±15 (5)	76± 14 (4)	n.d.
Rice	20± 3 (4)	n.d.	590± 72 (3)
Wheat germ	35± 4 (6)	187± 61 (5)	390±103 (3)
Spinach	104±25 (8)	689±244 (6)	>3,000
Yeast	80±12 (8)	62± 7 (4)	1,100±310 (3)

Values are means±SE of (n) determinations. n.d., not determined.

bean and rice to the three γ-glutamylcysteinyl tripeptides in comparison to the affinities of purified GR's from spinach, wheat germ and yeast (Table 2). The K_m values of all enzymes for GSSG are in the range of 20–104 μM and are similar to those determined for other GR's (Mahan and Burke 1987). The homologues hGSSG and HMGSSG are also reduced by the GR's from all species tested but with strongly varying efficiencies (Table 2). Whereas the affinity of bean GR for hGSSG and GSSG is nearly the same, the GR's from spinach and wheat show a 5–7 fold higher affinity for GSSG. HMGSSG turned out to be an inadequate substrate for all GR's, but the rice and wheat germ enzymes show a significant higher affinity to this glutathione homologue than the spinach and yeast enzymes. Thus, the GR's of the different plants seem to be adapted to the tripeptide species present in the respective plants.

The maximal velocity at saturating substrate concentrations, determined for GSSG and for hGSSG by the Lineweaver-Burk plot, is nearly the same for both disulfides with all GR's: the ratio of V_{max} for hGSSG to V_{max} for GSSG is 0.75 for spinach, 0.80 for wheat germ, 0.91 for yeast and 0.86 for bean. Therefore GSSG can be used as a

Table 3 Activities of AsA peroxidase, MDA reductase, DHA reductase and GR in leaf extracts (Leaf) and chloroplasts (Chlp) from five plant species

Species		AsA peroxidase	MDA reductase	DHA reductase	GR
Wheat	Leaf	10.7	1.95	3.45	1.30
	Chlp	1.23	0.47	1.02	0.73
	Chlp (%)	13± 3	28±4	34±10	65± 3
Bean	Leaf	5.64	1.98	0	0.36
	Chlp	1.18	1.01	0	0.21[a]
	Chlp (%)	28± 3	68±2	0	78±14
Soybean	Leaf	2.72	1.85	0.14[a]	0.13[a]
	Chlp	3.13	0.53	0	0
	Chlp (%)	132±11	39±6	0	0
Spinach	Leaf	6.22	3.08	1.70	1.74
	Chlp	3.56	1.01	0.52	1.40
	Chlp (%)	64± 4	36±2	34± 2	89± 4
Pea	Leaf	5.09	2.07	0.49	1.06
	Chlp	3.46	0.74	0.26	0.90
	Chlp (%)	71± 7	37±4	55± 6	88± 4

Activities are given in μmol mg Chl^{-1} min^{-1}, and means of at least three independent experiments. SE of the enzyme activity values was less than one third of the mean values, except for the values marked by [a], where it was less than two-thirds of the mean values. The procentual localization of each enzyme in the chloroplasts was calculated with consideration of the intactness of the chloroplasts used for enzyme extraction. 0, not detectable.

substrate for assaying the GR from bean and probably other hGSH containing plants.

Activities of the enzymes of the ascorbate/glutathione cycle—The activities of the four soluble enzymes concerned with H_2O_2 scavenging, determined in the leaf extracts as well as in the chloroplasts of spinach and pea (Table 3), show a good agreement with published results (Hossain et al. 1984, Gillham and Dodge 1986). AsA peroxidase was found at 3- to 10-fold higher activity in chloroplasts than other enzymes. The activities of MDA reductase and DHA reductase will be nearly the same at the basic pH of the chloroplast stroma (DHA reductase activity under assay conditions at pH 6.5 is only 40% that at pH 7.8 according to Asada (1984)) and therefore do not allow a prediction, by which way AsA is regenerated in these species. The localization is between 34 and 89% in chloroplasts for all enzymes. The results for the wheat leaf and chloroplast enzymes are similar to those found with spinach and pea. With bean and soybean, however, there is a significant difference: both species have no or little activities of DHA reductase and a low activity of GR. Since both species are homoglutathione containing plants, the enzyme activities were also measured using homoglutathione as the substrate in the assay, but the results were the same.

Light-dependent H_2O_2 reduction—The ability for light-dependent reduction of H_2O_2 was studied with intact chloroplasts of bean, soybean and wheat in comparison to spinach and pea. The intactness of the chloroplasts was between 71% and 95% (Table 1). The activity of the Hill reaction of all chloroplasts assayed after lysis of intact chloroplasts and using ferricyanide as electron acceptor was in the same magnitude, ranging from 3.5–4.8 μmol O_2 mg Chl^{-1} min^{-1}. In the dark the O_2-evolution rate after injection of 50 μM H_2O_2 was below 0.07 μmol O_2 mg Chl^{-1} min^{-1} with all chloroplasts indicating that catalase was nearly absent in the chloroplast preparations.

For four species the (light plus H_2O_2)-dependent O_2-evolution rate, determined at 50 μM H_2O_2, proved to be dependent on the presence of AsA in all media used for the isolation of the chloroplasts. With the wheat and pea chloroplasts the rates were very low without the addition of AsA (0.2 and 0.1 μmol mg Chl^{-1} min^{-1}, respectively) but reached maximal values already at 1 mM AsA (1.6 and 1.2 μmol mg Chl^{-1} min^{-1}, respectively). No light-dependent reduction of H_2O_2 was detectable with the bean and soybean chloroplasts in the absence of AsA. The AsA concentration in the isolation media had to be increased up to 5 mM in order to reach the maximal rate of 0.6 μmol mg Chl^{-1} min^{-1} for both species. Only with the spinach chloroplasts the ability for light-dependent reduction of H_2O_2 proved to be independent from the presence of AsA. The O_2-evolution rate was 1.3 μmol mg Chl^{-1} min^{-1}, which is similar to the rate of 0.8–1.2 μmol mg Chl^{-1} min^{-1} determined by Asada and Badger (1984) for this species.

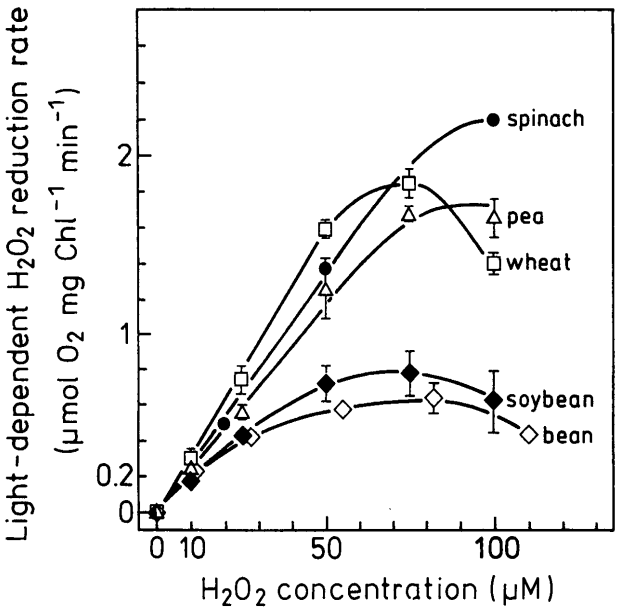

Fig. 1 Effect of H_2O_2 concentration on light-dependent O_2-evolution in intact chloroplasts of five plant species. Intact chloroplasts were isolated with 5 mM AsA (bean and soybean), with 1 mM AsA (pea and wheat) or without AsA (spinach) in the isolation medium. The chloroplasts (19–34 μg Chl ml^{-1} assay medium) were preincubated in the cuvette for 3 min in the light. The reaction was started by injection of H_2O_2 at the indicated concentrations and the initial rates of O_2-production were determined. These rates were corrected for the low O_2-evolution rates occurring after H_2O_2 injection to the chloroplasts in the dark.

The light-dependent H_2O_2 reduction rates after addition of various concentrations of H_2O_2 to the isolated chloroplasts are shown in Fig. 1. For spinach and pea chloroplasts, the reduction rate was saturated at nearly 100 μM H_2O_2. With the wheat chloroplasts the rate was maximal at 75 μM H_2O_2 and decreased at higher concentrations. All three species exhibited a comparable activity of (light plus H_2O_2)-dependent O_2-evolution. This activity was much lower for the bean and soybean chloroplasts and reached rates of 0.5–0.8 μmol O_2 mg Chl^{-1} min^{-1} at optimal H_2O_2 concentrations. At 10 μM H_2O_2, however, the O_2-evolution rate was the same for all species: 0.2–0.3 μmol O_2 mg Chl^{-1} min^{-1}. In Percoll-purified, intact spinach chloroplasts the H_2O_2 concentration has been determined to be below 1 μM (Nakano and Asada 1980). Thus all species seem to possess sufficient capacities for H_2O_2 reduction at physiological concentrations.

The expected stoichiometry in the ascorbate/glutathione H_2O_2 scavenging system, which uses one mol of NADPH for the reduction of one mol H_2O_2, is 0.5 mol O_2 evolved per mol of H_2O_2 consumed. This stoichiometry is achieved in the case of the spinach, pea and wheat chloroplasts: the injection of 25 nmol H_2O_2 leads to the evolution

of 12.8±0.4, 13.0±1.0 and 12.8±2.3 nmol O_2, respectively. With the bean and soybean chloroplasts, the evolution of O_2 after the injection of 25 nmol H_2O_2 ceased after the evolution of 8.4±1.5 and 9±1 nmol O_2, respectively. We conclude that in these chloroplasts part of the H_2O_2 is scavenged by other enzymatic or chemical mechanisms which do not use photoreductants.

Discussion

Intact chloroplasts of bean, soybean, wheat and pea show a light-dependent H_2O_2 reduction only if they were isolated in the presence of an adequate concentration of AsA in all isolation media. This result is explained by the fact that the chloroplastic AsA peroxidase which reduces H_2O_2 is readily inactivated in the absence of AsA (Hossain and Asada 1984). Although the AsA concentration of chloroplasts is relatively high (10 mM for spinach, Hossain and Asada 1984, 20–25 mM corresponding to 0.6 μmol mg Chl^{-1} for pea, Gillham and Dodge 1986), this pool may be oxidized or lost by leakage during the isolation of the chloroplasts. Spinach chloroplasts are able to take up AsA at a significant rate (ca. 0.2 μmol AsA mg Chl^{-1} min^{-1} at 20°C and 10 mM AsA, Anderson et al. 1983) and the efflux rate may be similar. The five species analysed presumably differ in the AsA contents of the leaves or chloroplasts, in the content of oxidizing cell metabolites, in the permeability of the chloroplast membrane to AsA or in the concentration dependence for the stabilizing effect of AsA on AsA peroxidase. Therefore, they need different concentrations of AsA in the isolation media to retain the H_2O_2 reduction ability in the light.

When isolated in the presence of AsA, bean and soybean chloroplasts have a light-dependent H_2O_2-reduction at physiological H_2O_2 concentrations as effective as spinach and pea chloroplasts do. The chloroplasts from bean and soybean contain 5–10% of the cellular hGSH pool. The affinity of bean GR to hGSSG is high enough to keep this thiol predominantly in the reduced state, since the K_m for hGSSG is several-fold lower than the tripeptide concentration in the chloroplasts. The activity of GR in the chloroplasts, however, is very low and DHA reductase is absent. Therefore it is questionable whether hGSH is involved in the light-dependent H_2O_2-reduction. These chloroplasts probably reduce H_2O_2 by the AsA peroxidase and MDA reductase pathway, whereas in wheat as well as in spinach and pea DHA reductase and GR participate in AsA regeneration. A light-dependent H_2O_2 reduction with photoreductants generated in the thylakoids but without activity of DHA reductase has recently been shown for some cyanobacteria (Miyake et al. 1991).

During the preparation of this paper results were published which show that spinach chloroplasts have a further H_2O_2 reduction system which is based on a thylakoid-bound AsA peroxidase and photoreduction of the MDA radical in the thylakoids (Miyake and Asada 1992). The authors propose that this membrane-bound system, which also is inactivated in the absence of AsA, is the primary scavenging system of H_2O_2 in the chloroplasts and that the stromal enzyme system plays a secondary role. It is possible that a similar membrane-bound system is responsible for part or all of the (light plus H_2O_2)-dependent O_2-evolution measured in our experiments.

The hGSH-content of the legume chloroplasts, however, will be of significance in situations of oxidative stress, because at high H_2O_2 production rates part of the MDA produced by the peroxidase reaction will escape from direct reduction and disproportionate to DHA, leading to a depletion of the AsA pool; this depletion may be prevented by nonenzymatic reduction of DHA by hGSH, which depends on the pH inside the chloroplasts and on the thiol concentration (Foyer and Halliwell 1977, Hausladen and Kunert 1990). It is well known that oxidative stress leads to an increase of the cellular thiol content and GR activity (see review by Smith et al. 1989, Ushimaru et al. 1992), which documents the essential role of both for the scavenging of H_2O_2 and other toxic oxygen species inside or outside the chloroplasts. This is also true for hGSH-containing plants. In soybean plants which were treated with the catalase inhibitor aminotriazol in order to achieve an accumulation of H_2O_2 derived from photorespiration, the hGSH content rised to levels four times higher than those occurring in non-photorespiring plants (Smith 1985). As a response to lipid peroxidation, induced by the herbicide acifluorfen, a several-fold increase of the tripeptide content of bean leaves as well as a 1.4-fold increase in GR activity was found (Schmidt and Kunert 1986). Other possible functions of hGSH and GR in the bean and soybean chloroplasts will be those postulated for GSH: regulation of the activity of various enzymes by controlling the degree of oxidation of their sulfhydryl groups and promotion of protein synthesis (Rennenberg 1982).

For hydroxymethylglutathione the function as a reductant is not yet clear. The high H_2O_2 reduction activity of the wheat chloroplasts is possibly related to the GSH content of the chloroplasts and to the high activity of all enzymes participating in the ascorbate/glutathione cycle, and not to low HMGSH contents. The HMGSH concentration in the chloroplasts is 0.2 mM and lies below the K_m-value of the wheat GR for HMGSSG. However, a thiol-disulfide exchange reaction between both tripeptides should be considered which directly would couple the oxidative status of GSH and HMGSH despite the low enzymatic reduction rate of HMGSSG by the wheat GR. Further experiments which show the influence of oxidative stress on the HMGSH content and thiol/disulfide status will probably help to understand the function of HMGSH in graminaceous plants.

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