AN OVERVIEW ON THE ROLE OF METHYLGLYOXAL AND GLYOXALASES IN PLANTS

Sudesh Kumar Yadav[†], Sneh L. Singla-Pareek and Sudhir K. Sopory*

Plant Molecular Biology,
International Centre for Genetic Engineering and Biotechnology,
Aruna Asaf Ali Marg, New Delhi, India
[†]Present address: Biotechnology Division, Institute of Himalayan Bioresource Technology, Palampur, Himachal Pradesh, India

SUMMARY

Methylglyoxal (MG) is a highly reactive cytotoxic α-oxoaldehyde compound and is formed endogenously via different enzymatic and non-enzymatic reactions. In plants MG is detoxified mainly via the glyoxalase system that is comprised of two enzymes, glyoxalase I and glyoxalase II. Glyoxalase I converts MG to *S*-D-lactoylglutathione by utilizing glutathione, while glyoxalase II converts *S*-D-lactoyl-glutathione to D-lactic acid, and during this reaction glutathione is regenerated. The presence and characterization of both glyoxalase I and II has been reported in many plants and the genes encoding these have been cloned and found to be regulated under various environmental conditions. In plants, MG has been found to be present during normal growth conditions and it accumulates to higher levels under various environmental stresses. Abiotic and heavy metal stresses induce reactive oxygen species (ROS) and MG. Overexpression of the glyoxalase pathway in transgenic tobacco and rice plants has been found to check an increase of ROS and MG under stress conditions by

* Author for correspondence:
Prof. S.K. Sopory
Plant Molecular Biology
International Centre for Genetic Engineering and Biotechnology
Aruna Asaf Ali Marg
New Delhi-110067, India
e-mail: sopory@icgeb.res.in

©Freund Publishing House Ltd., 2008

Authenticated | xajibim@mail.ru
Download Date | 6/16/12 12:10 AM

maintaining glutathione homeostasis and antioxidant enzyme levels. There is also evidence that in addition to glyoxalase, other pathways, such as the aldose reductase pathway, may also be involved in MG detoxification in plants. To unravel the role of MG and the glyoxalase pathway in signal transduction during environmental stress conditions in plants is a topic of future research interest. In this paper we review work on plant glyoxalases especially with respect to their role under abiotic stresses.

KEY WORDS

methylglyoxal, glyoxalase enzymes, abiotic stress, plants

INTRODUCTION

Albert Szent-Györgyi and co-workers were among the first to study the biological effects of methylglyoxal. In their first study they observed a reversible inhibition of *E. coli* cell growth upon incubation with 1 mM methylglyoxal (MG), and this concentration showed maximum inhibitory effect after 6 hours /1/. In addition, ascites tumors in albino mice were susceptible to the cytostatic effects of MG at concentrations >1.0 mM /2/. Based on these observations, Szent-Györgyi and others suggested that MG was a physiological growth-inhibiting substance, and with its biological antagonist glyoxalase I, it controlled cell growth. This was reflected in a promine/ retine concept which over the years has not stood the test of experimentation (see /3/). Since MG inhibited protein synthesis /4/, Szent-Györgyi /5/ proposed that MG may change the electronic nature of proteins by binding and modifying susceptible amino acid residues, and thereby influencing cellular function. However, later reports document that it was *S*-D-lactoylglutathione rather than MG modifying the proteins. However, cellular concentrations of methylglyoxal from 0.1-2 μM were found to be of limited physiological significance. Ohmori *et al.* /6/ and Thornalley *et al.* /7/, however, gave the first indication that the growth inhibition induced by high concentrations of MG might be pharmacologically useful in cancer chemotherapy.

Several harmful compounds are generated upon exposure of plants to environmental stresses. The formation of D-amino acids under such

Authenticated | xajibim@mail.ru
Download Date | 6/16/12 12:10 AM

adverse conditions has been reported /8/. Glyoxal, which is a reactive α-oxoaldehyde and a physiologically active metabolite, is formed by lipid peroxidation, ascorbate autoxidation, oxidative degradation of glucose, and degradation of glycated proteins during normal physiological conditions /9/. It has now been shown that MG and 3-deoxyglucosone are capable of inducing cellular damage directly via protein glycation leading to the production of advanced glycation end-products (AGEs) which, in turn, may also contribute to cytotoxicity. MG also modifies DNA by reacting with guanyl residues of DNA forming imidazopurinone adducts /10/. These modifications are sufficient to induce loss of cell viability and ultimately lead to death of an organism /11/. Biosynthesis of MG and its role and catabolism via the glyoxalase pathway have been studied in some detail in yeasts and humans (see /12/). It is only in recent years that its presence and functions have been studied in plants.

The glyoxalase system is a ubiquitous pathway and exists in prokaryotes and eukaryotes for the detoxification of highly reactive ketoaldehydes. Glyoxalase I (EC 4.4.1.5.; lactoylglutathione methylglyoxal lyase) and glyoxalase II (EC 3.1.2.6.; hydroxyacylglutathione hydrolase) catalyze the degradation of 2-ketoaldehydes into the corresponding 2-hydroxy acids using glutathione as cofactor /10/. The main physiological function of the glyoxalase system is probably the detoxification of MG. Presently our understanding of MG synthesis and degradation in plants is limited and there seem to be different pathways (Fig. 1). In this paper, we present the current status of understanding of MG formation and its detoxification in plants, and the possible role of MG and the glyoxalase system in plants especially under stress environment.

METHYLGLYOXAL DETOXIFICATION IN PLANTS

Glyoxalase pathway

The glyoxalase metabolic pathway is present in the cytosol of cells and cellular organelles, particularly mitochondria. It is found in prokaryotic as well as eukaryotic organisms and is thought to be ubiquitous. The glyoxalase pathway was discovered independently by two groups in the same year /13,14/. Hopkins and Morgan /15/ showed its wide distribution among living organisms. The widespread

Authenticated | xajibim@mail.ru
Download Date | 6/16/12 12:10 AM

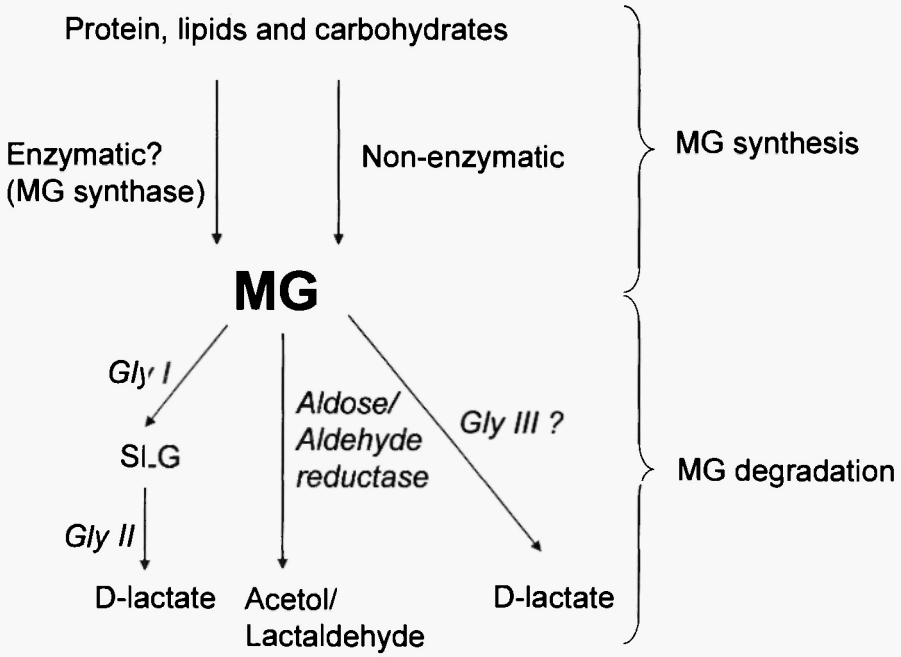

Fig. 1: Schematic representation of methylglyoxal (MG) synthesis and degradation in plants. MG is formed from proteins, lipids and carbohydrates through enzymatic (not yet confirmed) and non-enzymatic mechanism(s). MG is detoxified in the system through the glyoxalase pathway as well as aldose/aldehyde reductase pathway. MG degradation by glyoxalase (Gly) III has been reported only in bacteria and its presence in plants has still to be confirmed. SLG = *S*-D-lactoylglutathione.

distribution and presence of the glyoxalase system in diverse groups of living organisms document its fundamental importance to biological life. Both glyoxalase I (gly I) and glyoxalase II (gly II) enzymes have been extensively studied in microbial and animal systems /16,17/ as compared to plants.

The existence of the glyoxalase pathway has been demonstrated in several plant species by reporting the presence of two enzymes, gly I and gly II, which have been purified and characterized from different plant species /18-23/. We describe below the work done on the two enzymes in plants and their putative functions in plant growth and under stress conditions.

Authenticated | xajibim@mail.ru
Download Date | 6/16/12 12:10 AM

Glyoxalase I

After the discovery of gly I activity in Douglas fir needles by Smits and Johnson /24/, its presence has been documented in several plant species, including maize, jute, wheat, *Sorghum* and *Aloe vera*, soybean, *Cicer, Amaranthus, Nicotiana, Brassica, Datura* and the pea /18,20,21,25-27/. Studies on tomato showed its presence in all types of cells and tissues; however, gly I was preferentially accumulated in phloem and sieve cells /28/. A detailed 2-D analysis of phloem proteins, followed by mass spectrometry, revealed that of the 45 proteins identified from phloem exudates of cucumber and pumpkin, gly I was also present /29/. Recently, gly I was also found to be conserved in tubers among different potato variants /30/. At the cellular level, the majority of gly I protein was localized in the cytosol except a small amount was found in association with membranes /31/.

The gene encoding the gly I enzyme has been isolated and characterized from various plant species, such as tomato /28/, chickpea /32/, *Brassica oleracea* /33/, *B. juncea* /34/, soybean /35/, wheat bran /36/ and rice /37/.

In contrast to the monomeric and homodimeric crystal structure of the gly I protein in microbial and mammalian systems, plant gly I protein exists primarily in oligomeric structure. The homodimeric crystal structure of the gly I protein from human /38-40/ and *E. coli* /41/ have revealed that each active site is composed of residues contributed by both subunits. This has been deciphered through the mode of binding of glutathione-based inhibitors /39,40/. The crystal structure has also helped in knowing the differential metal dependence of typical eukaryotic and prokaryotic gly I enzymes. The metal choice was found to be based on the structural requirement of octahedral coordination to stabilize the transition state /41/. The gly I enzyme from *Brassica* and soybean exists as a dimer. *Brassica* gly I has a molecular mass of 58 kDa with two subunits of 27-29 kDa, while soybean gly I is 60 kDa with two subunits of 26-29 kDa /20,21,42/. The *A. vera* gly I exists as a monomer with a molecular mass of 44 kDa /18/. The pI value of plant gly I is very similar to animal gly I, which ranges from 4.7-5.1 /21/. However, *A. vera* gly I has a higher pI of 7.8 /18/. The 37 kDa wheat enzyme belongs to a group of monomeric glyoxalases and is composed of two similar halves, each representing the full-length human gly I enzyme.

Authenticated | xajibim@mail.ru
Download Date | 6/16/12 12:10 AM

Similar to other organisms, plant gly I follows steady state kinetics over a restricted range of hemithioacetal substrate, i.e. 0-0.67 mM. The K_m value for hemithioacetal is 460 μM in soybean /21/ and 1,400 μM in *A. vera* /18/. Biochemical characterization of gly I has further indicated that its active site has binding affinity for zinc ion and hemithioacetal, and the His residue might be important for its catalytic activity, as it was found to be present at the active site of *B. juncea* gly I protein /31/.

Glyoxalase II

Glyoxalase II catalyzes the second step in MG detoxification and forms D-lactate in the system. It has now been shown that D-lactate in plants is metabolized inside mitochondria by a flavoprotein, a putative D-lactate dehydrogenase /43/. The native gly II protein has been purified from *Zea mays* /44/, *Aloe vera* /18/ and spinach leaves /19/ for functional analysis. The gene encoding gly II has been cloned and recombinant protein purified and characterized from *Arabidopsis thaliana* /45/, *Brassica* /46/ and rice /23/.

Unlike animal gly II, which has basic pI, plant gly II has acidic pI. The purified gly II (26 kDa) from *Zea mays* /44/, multiple forms from *A. vera* /18/ and spinach leaves /19/ have acidic pI ranging from 4.5-6.2. However, the mitochondrial isoform in rice has a pI value in the basic range, i.e. 8.08 /23/. The values of kinetic constants using several glutathione thiolesters as substrates are similar for the enzymes from cytosol and mitochondria /19/. In plants, the presence of peculiar forms of gly II in mitochondria have been reported which are similar to those shown earlier in mammalian systems. The structural and functional similarities between gly II from plants and from human tissues further suggest a common evolutionary origin for this enzyme /47/. The K_m of *Brassica juncea* gly II with *S*-D-lactoylglutathione as substrate was determined to be 120 μM /46/, while rice gly II has a lower K_m of 61 μM /23/.

Two gly II isoforms, mitochondrial and cytosolic, were identified from *Arabidopsis*. Detailed structural studies of mitochondrial gly II have shown that it can accommodate a number of different metal centers, but the main ones are Fe(III) and Zn, and it does not specifically bind Mn /48/. The cytosolic gly II was shown to bind Zn, Fe or even Mn, and showed positive cooperativity in metal binding /49/. Northern blot analysis shows that the two genes are differentially

Authenticated | xajibim@mail.ru
Download Date | 6/16/12 12:10 AM

expressed. The mitochondrial isozyme is most abundant in roots, while the cytoplasmic isozyme is highest in flower buds. The identification of gly II isozymes that are differentially expressed suggests that they may play different roles in the cell /45/. The cytoplasmic form of *Arabidopsis* gly II contains an iron-zinc binuclear metal center that is essential for activity. Both metals participate in substrate binding, transition state stabilization, and the hydrolysis reaction. Subtle alterations in the geometry and/or electrostatics of the binuclear center have profound effects on the activity of the enzyme /50/. Adjacent residues also help in substrate binding in the gly II protein. This molecular information have been used to design gly II inhibitors, such as di-FMOC (9-fluorenylmethoxycarbonyl) and di-Cbz compounds, which showed K_i values of 0.89 ± 0.05 and 2.3 ± 0.5 μM, respectively /51/.

As in *Arabidopsis*, the gly II from *Brassica juncea* also showed a conserved THHHXDH domain which is involved in zinc binding /46/. The amino acid sequence of *Brassica juncea* gly II showed 92% and 56% identity with *Pennisetum* and rice gly II, respectively, while it showed only 30% identity with human gly II. The presence of N-terminal mitochondrial target peptide in the *Brassica juncea* gly II provides evidence for its mitochondrial isoform. Similarly, rice gly II amino acid sequence shared 57% identity with *Arabidopsis*, whereas identity with human and yeast gly II was 30% and 24%, respectively. It also contains the highly conserved metal binding motif (THHHXDH), involving a cluster of histidines centered around position 135 among plants and 60 among yeast and humans. About 55 residues away from the conserved histidine cluster (THHHXDH), another highly conserved sequence G/CHT is also present, as exists in all other gly II sequences /23/.

Other pathways

There are other routes through which MG could be detoxified in the plant system. MG contain oxo and aldal groups and hence can undergo oxidation or reduction reactions. Therefore, the enzymes involved in oxido-reductions could catalyze the conversion of MG. Reduction of MG through this pathway is expected to result in two products, either acetol or lactaldehyde. Oxido-reductases and dehydrogenases are capable of performing these reactions. The reductase family of enzymes involving in MG detoxification is represented as

Authenticated | xajibim@mail.ru
Download Date | 6/16/12 12:10 AM

ALR1, ALR2 and ALR3. These are aldehyde reductase (alcohol: NADP-oxido-reductase, EC.1.1.1.2.), aldose reductase (alditol:NADP-oxido-reductase, EC.1.1.1.21.) and carbonyl reductase (EC.1.1.1.184.), respectively /52/. All ALRs have been shown to possess broad substrate specificity and are located in the cytosol. They can potentially be involved in MG detoxification in plants.

It was found that overexpression of a novel alfalfa aldose/aldehyde reductase (*MsALR*) in tobacco plants showed tolerance against oxidative agents and drought stress. The plants showed lower concentrations of reactive aldehydes, including MG, generated inside the cell under stress /53,54/. Here the mechanism of tolerance to stress, as discussed below for glyoxalase transgenics, could be different, as ALR uses NADPH instead of GSH for the reduction of reactive aldehydes such as 4-hydroxynon-2-enal, MG, etc.

The gly III enzyme is known to catalyze the detoxification of MG in a single step and in a GSH-independent manner. This enzyme has so far been identified in *E. coli* and used for MG detoxification. Several other bacteria are now known to show gly III activity /55/. MG detoxification is also catalyzed by glyoxal oxidase that produces H_2O_2. This has been characterized from *Phanerochaete chryssosporium* /56/. To date, gly III and glyoxal oxidase are not known in plant systems. Pyruvate dehydrogenase has also been shown to catalyze MG detoxification /57/. This enzyme is found in abundance in plants, but its role in MG degradation is not well studied in any living organism including plants.

REGULATION OF THE GLYOXALASE PATHWAY

The regulation of glyoxalase pathway enzymes has not been well studied at the molecular level; however, few physiological and biochemical studies have been reported. Enzymes of the glyoxalase system in plants are under the regulation of developmental and hormonal changes and environmental influences such as light and salinity. The activity of gly I showed linear progression with development of shoots and roots of pea seedlings. Cell division and proliferation further modulate the activity of gly I. Cell division and development in plants are in direct correlation with gly I activity. Indole acetic acid (IAA) stimulated growth and development as well as gly I activity in the pea, while colchicine and vinblastine, inhibitors of cell division,

Authenticated | xajibim@mail.ru
Download Date | 6/16/12 12:10 AM

decreased gly I activity /20/. This suggests that gly I is important for growth and development of plants. Further evidence for this was shown by using inhibitors of gly I that arrested cell division. Spermidine, an inducer of cell growth, also enhanced gly I activity /58, 59/. *Brassica* cultures may be induced to undergo differentiation by lack of hormones in basal growth medium, and dedifferentiated by the addition of 1-naphthaleneacetic acid and benzyladenine. The activity of gly I decreased by approximately 50% during differentiation, suggesting the inhibitory role of 1-naphthaleneacetic acid and benzyladenine on gly I activity /60/. Similarly, gly II also showed differential expression, as it exists in two isoforms in the mitochondria and in the cytosol. These isoforms might have different roles in these compartments /45/.

Phytohormones, such as 6-benzylaminopurine (BAP), IAA and kinetin, play a significant role in cell growth, and were shown to affect gly I activity. These hormones increased gly I activity along with cell numbers in pea and *Datura* and *Nicotiana* calluses /59,60/. Similarly, *C. capsularis* and *Amaranthus* calluses showed an inverse relationship between differentiation and the activity level of gly I /27/. Auxin supplement enhanced cell growth and the activity of both gly I and gly II in soybean /21/. The mechanism by which hormones affect glyoxalase activity is not clear. In a recent study it was found that one of the proteins amongst 60 that were phosphorylated by calcium-dependent protein kinases (CDPKs) in response to gibberellic acid in rice was gly I /61/. Analysis of phosphoproteome under salinity stress in rice has indicated gly I as one of the upregulated phosphoproteins /62/. In another study it was shown that SNRK2.8, a member of the sucrose non-fermenting related kinases, can phosphorylate gly I /63/. Whether phosphorylation of gly I affects its activity is not yet known.

The role of light in growth and development of plants has been known for a long time. However, glyoxalase pathway enzymes are also found to respond to light signals. Red and blue light exposure increases gly I activity in dark-grown *Amaranthus* calluses, indicating gly I expression is under stringent regulation by photoreceptors in plants /26/. Calcium and calmodulin exposure involved in cell proliferation also activated gly I. Correlation between cell proliferation and gly I activity was further confirmed by using lithium chloride that inhibited both processes /64/. These studies are indicative of calcium-mediated light signals in gly I activation. Not only light but a number

Authenticated | xajibim@mail.ru
Download Date | 6/16/12 12:10 AM

of other exogenous factors, such as hypoxia, temperature shock, and water stress, transduce their signal through Ca^{2+} that binds to specific target proteins, including kinases, which in turn could activate gly I. It is also possible that Ca^{2+} binds to calcium binding proteins such as calmodulin, and modulates the activity of gly I along with various other proteins /65/. It was found that *in vitro* gly I activity was stimulated by exogenous addition of calmodulin /66/.

INVOLVEMENT OF THE GLYOXALASE PATHWAY IN STRESS ENVIRONMENTS

Indication towards the role of gly I in abiotic stress came from studies on tomato. Exposure of tomato to salt, mannitol and abscisic acid showed an increase in gly I activity levels in roots, stems and leaves /28/. This was followed by a more detailed study where a cDNA from *Brassica* was overexpressed in tobacco and the transgenic plants were found to tolerate higher concentrations of NaCl /34/. Johansen *et al.* /36/ found that wheat bran gly I showed very high similarity to the translated sequence of a RNA transcript induced by desiccation in a resurrection plant, *Sporobulus stapfianus*, thus suggesting a role of gly I in dehydration or rehydration. Cell lines of *A. hypogea* also showed gly I activation upon exposure to high salt and herbicide (glyphosate) /67/. Conclusive evidence for the role of both gly I and gly II in stress tolerance came from transgenic studies in which both genes were overexpressed in tobacco; such plants showed a high level of tolerance to salinity and heavy metal stresses /22,68/. It was found that drought, salt, heat, cold and zinc stress also enhanced gly II transcript expression in *Brassica* and rice /23,46/. Overexpression of gly II was also found to confer stress tolerance in rice /69/. These studies have clearly implicated a role of the glyoxalase pathway in plants during stress exposure.

Mechanism of glyoxalase-mediated stress tolerance

MG is a cytotoxic and very harmful molecule to the cellular system if overproduced. It was shown recently that MG levels in plants increase in response to abiotic stresses /70,71/. Both leaves and roots of plants, including *Oryza sativa*, *Pennisetum glaucum*, *Nicotiana tabacum* and *Brassica juncea*, had increased levels of MG when

Authenticated | xajibim@mail.ru
Download Date | 6/16/12 12:10 AM

exposed to stress conditions. A certain basal concentration of MG is essential for normal physiological functions, which is species specific. The basal level of MG was found to be the same in leaves and roots, except in rice, in which its level was lower in roots compared to leaves. However, *Pennisetum* and tobacco showed lower levels of MG than rice and *Brassica* under normal conditions. Among these plants, MG levels ranged from 40-75 μM under non-stress conditions. However, under stress the level of MG was enhanced up to sixfold. Salt stress raised its level from 75 to 200 μM, while drought stress increased its level up to 300 μM in leaves /70/. The range of MG reported is very wide in nature, with as high as 300 μM MG in cultured Chinese hamster ovary cells and as low as 0.3 μM in yeast and in other animal systems /72,73/. Plants in general have higher levels of MG. Furthermore, the accumulation of MG under drought, salinity, and cold stress conditions in shoot and root tissues of two varieties of rice (PB1 and IR64) suggests that this could be a universal response in plants /70/.

To protect plants from the toxic effect of MG under stress, the system needs an efficient detoxification mechanism. This could be the reason that by overexpressing the enzymes of the glyoxalase pathway, transgenic plants could survive under stress environments /22,68,69/. In addition to a MG detoxification mechanism, it was found that glutathione, that is regenerated by the glyoxalase system, may play an important role during stress conditions /70,74/.

The independent role of glutathione has been shown in providing tolerance to various stresses in plants. Glutathione is one of the very important reducing cellular equivalents involved in various reducing activities in addition to providing buffering capacity to living cells. In plants, glutathione is present in two forms, viz. reduced glutathione (GSH) and oxidized glutathione (GSSG). Under normal physiological conditions, plants have ~90% GSH to GSSG ratio. However, under stress conditions, this ratio declines and glutathione homeostasis is disturbed in the system. Since reduced glutathione is involved in reducing the harmful levels of ROS by direct quenching and through the glutathione-ascorbate cycle, decrease in its concentration under stress leads to an increase in ROS. The high level of ROS then induces the production of several reactive carbonyl compounds, including MG.

It was found that plants overexpressing glyoxalase enzymes maintain the GSH/GSSG ratio and this may protect the plants from

Authenticated | xajibim@mail.ru
Download Date | 6/16/12 12:10 AM

abiotic stress induced oxidative stress /74/. However, the mechanism of glutathione homeostasis maintenance in transgenic plants is not known. Two possibilities were assumed: one is recycling of GSH by the glyoxalase pathway, and γ-glutamylcysteine synthetase might not be undergoing feedback inhibition, thus synthesis of glutathione continues. Secondly, the metabolic intermediates of the glyoxalase pathway might be acting as signal molecules to regulate the enzymes of glutathione biosynthesis. Since the role of *S*-D-lactoylglutathione in GSH level enhancement has been observed /17/, the possibility of other metabolites of the glyoxalase pathway, such as MG or D-lactate in regulating GSH homeostasis cannot be ruled out.

In addition to the role of the glyoxalase pathway in stress tolerance of plants through direct MG detoxification and maintenance of the GSH/GSSG ratio, it may also be involved in the regulation of various antioxidative enzymes that utilize GSH. Transgenic plants over-expressing glyoxalase genes maintain higher levels of their basal antioxidative enzymes, such as glutathione reductases (GR), glutathione-*S*-transferase (GST), glutathione peroxidase (GPx) and ascorbate peroxidase (APx), that further increase upon salt stress exposure /74/. Higher GST activity in plants could provide tolerance by detoxifying toxic radicals generated due to stress exposure, and scavenging of ROS by APx increases monodehydroascorbate (MDHA) and dehydroascorbate (DHA) to reduced ascorbate (ASH). For the continuation of scavenging process by APx, these should be available in their reduced form and, therefore, a regeneration system comprised of MDHA reductase, DHA reductase and GR brings about the reduction of oxidized ASH using GSH /75/. GPx is involved in the removal of reactive peroxides formed inside the cell during stress exposure. Therefore, higher levels of GPx provide tolerance to plants by degrading lipid peroxides which could otherwise damage the cell membrane. GR is the 'master enzyme' of GSH-utilizing antioxidative enzymes. GR catalyzes the reduction of GSSG to GSH in a NADPH-dependent reaction and its higher activity could result in higher GSH/GSSG ratio in plants, as seen in the case of glyoxalase transgenic plants. Maintenance of higher GSH/GSSG ratio due to high GR activity in these plants could be responsible for higher activity of other antioxidative enzymes /74/. Presently it is not known whether the increase in the activity of antioxidative enzymes and the antioxidant, glutathione, in glyoxalase-overexpressing transgenic plants exposed to

Authenticated | xajibim@mail.ru
Download Date | 6/16/12 12:10 AM

salt stress is due to enhanced expression of genes controlling the biosynthesis of these enzymes and/or increased activation of pre-existing enzyme pools. However, a high level of ROS inducing the expression of such enzymatic genes has been shown in various other studies /76/.

The mechanism shown above may be true for many abiotic stresses. However, in the case of salinity stress one needs to check how glyoxalase overproduction could also take care of higher Na^+ levels in plants. Similarly, one also needs to explain tolerance to heavy metal stresses conferred by the glyoxalase pathway. It was shown by Singla-Pareek *et al.* /68/ that GSH could be used to generate phyto-chelatins which form complexes with metal ions and target them to the vacuoles. Metal induction of glyoxalases has been shown in some systems /34,46/. Recently a study of the proteome following copper stress revealed that 25 proteins were differentially expressed, and one of these proteins was found to be gly I /77/.

FUTURE PERSPECTIVES

Knowledge of the glyoxalase pathway and its role in methylglyoxal detoxification has considerably expanded over the years, at least in animal systems. However, detailed work on plants has been undertaken only recently. The exact mechanism by which MG is primarily produced is not known but most likely it is through a non-enzymatic route. This area needs further investigation. More studies are needed to determine the range of MG levels in plants and its role in normal growth and development. Our earlier studies have given correlative evidence on the role of the glyoxalase system in cell proliferation and differentiation. Further studies are needed to elucidate these roles with more direct experimentation.

Recently, increased MG levels were observed when plants were exposed to abiotic and heavy metal stresses. As reported in animal systems, also in plants a high level of MG causes harm in growth and development. It was found that MG levels could be maintained during exposure to stress by overexpressing glyoxlase I and II through a transgenic approach. Therefore, engineering of the glyoxalase pathway, or other MG degrading pathways, should turn out to be an important strategy in ameliorating the effect of abiotic and heavy metal stresses. This needs to be tested in other crops and also under

Authenticated | xajibim@mail.ru
Download Date | 6/16/12 12:10 AM

natural stress environments. The mechanism of this protection seems to be through direct degradation of increased MG as well as by controlling ROS level by maintaining GSH redox homeostasis and increasing the levels of antioxidative enzymes. Nevertheless, more detailed studies on these and other metabolomic changes that occur in glyoxalase-overexpressing transgenic plants are required. In view of recent studies in yeast, it would be worthwhile to investigate whether MG might be involved in signal transduction pathways also in plants.

REFERENCES

1. Együd LG, Szent-Györgyi A. Cell division, SH, ketoaldehydes, and cancer. Proc Natl Acad Sci USA 1966; 55: 388-393.
2. Együd LG, Szent-Györgyi, A. Cancerostatic action of methylglyoxal. Science 1968; 160: 1140.
3. Kalapos MP. On the promine/retine theory of cell division: now and then. Biochim Biophys Acta 1999; 1426: 1-16.
4. Együd LG, Szent-Györgyi A. On the regulation of cell division. Proc Natl Acad Sci USA 1966; 56: 203-207.
5. Szent-Györgyi A. The living state and cancer. Proc Natl Acad Sci USA 1977; 74: 2844-2847.
6. Ohmori S, Mori M, Kawase M, Tsuboi S. Determination of methylglyoxal as 2-methylquinoxaline by high-performance liquid chromatography and its application to biological samples. J Chromatogr B 1987; 414: 149-155.
7. Thornalley PJ. Modification of the glyoxalase system in human red blood cells by glucose in vitro. Biochem J 1988; 254: 751-755.
8. Erikson O, Hertzberg M, Näsholm T. A conditional marker gene allowing both positive and negative selection in plants. Nat Biotechnol 2004; 22: 455-458.
9. Shangari N, O'Brien PJ. The cytotoxic mechanism of glyoxal involves oxidative stress. Biochem Pharmacol 2004; 68: 1433-1442.
10. Thornalley PJ. Glutathione-dependent detoxification of α-oxoaldehyde by the glyoxalase system: involvement in disease mechanisms and antiproliferative activity of glyoxalase I inhibitors. Chem Biol Interact 1998; 111-112: 137-151.
11. Booth IR, Ferguson GP, Miller S, Li C, Gunasekera B, Kinghorn S. Bacterial production of methylglyoxal: a survival strategy or death by misadventure? Biochem Soc Trans 2003; 31: 1406-1408.
12. Kalapos MP. Methylglyoxal in living organisms: chemistry, biochemistry, toxicology and biological implications. Toxicol Letts 1999; 110: 145-175.
13. Neuberg C. The destruction of lactic aldehyde and methylglyoxal by animal organs. Biochem J 1913; 49: 502-506.
14. Dakin HD, Dudley HW. An enzyme concerned with the formation of hydroxyl acids from ketonic aldehydes. J Biol Chem 1913; 14: 155-157.

Authenticated | xajibim@mail.ru
Download Date | 6/16/12 12:10 AM

15. Hopkins FG, Morgan EJ. On the distribution of glyoxalase and glutathione. Biochem J 1945; 128: 321-329.
16. Rhee HI, Murata K, Kimura A Molecular cloning of *Pseudomonas putida* glyoxalase I gene in *E. coli*. Biochem Biophys Res Commun 1987; 147: 931-938.
17. Thornalley PJ. The glyoxalase system: new developments towards functional characterization of a metabolic pathway fundamental to biological life. Biochem J 1990; 269: 1-11.
18. Norton SJ, Talesa V, Yuan WS, Principato GB. Glyoxalase I and glyoxalase II from Aloe vera: purification, characterization and comparison with animal glyoxalases. Biochem Int 1990; 22: 411-418.
19. Talesa V, Rosi G, Contenti S, et al. Presence of glyoxalase II in mitochondria from spinach leaves: comparison with the enzyme from cytosol. Biochem Int 1990; 22:1115-1120.
20. Deswal R, Chakravarty TN Sopory SK. The glyoxalase system in higher plants: regulation in growth and differentiation. Biochem Soc Trans 1993; 21: 527-530.
21. Paulus C, Knollner B, Jacobson H. Physiological and biochemical characterization of glyoxalase I, a general marker for cell proliferation, from a soybean cell suspension. Planta 1993; 189: 561-566.
22. Singla-Pareek SL, Reddy MK, Sopory SK. Genetic engineering of the glyoxalase pathway in tobacco leads to enhanced salinity tolerance. Proc Natl Acad Sci USA 2003; 100: 14672-14677.
23. Yadav SK, Singla-Pareek SL, Kumar M, et al. Characterization and functional validation of glyoxalase II from rice. Protein Expr Purif 2007; 51: 126-132.
24. Smits MM, Johnson MA. Methylglyoxal: enzyme distributions relative to its presence in Douglas-fir needles and absence in Douglas-fir needle callus. Arch Biochem Biophys 1981; 208: 431-439.
25. Ramaswamy O. Role of glyoxalase I in higher plants. Jawaharlal Nehru University, New Delhi: Ph.D. Thesis, 1982.
26. Chakravarty TN, Sopory SK. Light stimulated cell proliferation and glyoxalase I activity in callus cultures of *Amaranthus paniculatus*. In: Nijkamp HJJ, Van-derplas LHW, Asrtrijk VJ, eds. Progress in Plant Cellular and Molecular Biology. Dordrecht: Kluwer Academic Press, 1990; 379-384.
27. Seraj ZI, Sarker AB, Islam AS. Plant regeneration in a jute species (*C. capsularis*) and its possible relationship with glyoxalase-I. Plant Cell Rep 1992; 12: 29-33.
28. Espartero J, Sanchez-Aguayo I, Pardo JM. Molecular characterization of glyoxalase I from a higher plant: upregulation by stress. Plant Mol Biol 1995; 29: 1223-1233.
29. Walz C, Giavalisco P, Schad M, Juenger M, Klose J, Kehr J. Proteomics of cucurbit phloem exudates reveals a network of defense proteins. Phytochemistry 2004; 65: 1795-1804.
30. Bauw G, Nielsen HV, Emmerson J, et al. Patatins, Kunitz protease inhibitors and other major proteins in tuber of potato cv. Kuras. FEBS J 2006; 273: 3569-3584.

Authenticated | xajibim@mail.ru
Download Date | 6/16/12 12:10 AM

31. Deswal R, Sopory SK. Biochemical and immunochemical characterization of *Brassica juncea* glyoxalase I. Phytochemistry 1998; 49: 2245-2253.
32. Romo S, Labrador E, Dopico B. The electronic plant gene register. Plant Physiol 1998; 117: 331.
33. Clugston SL, Daub E, Honek JF. Identification of glyoxalase 1 sequences from *Brassica oleracea* and *Sporobolus stapfianus*: evidence for gene duplication events. J Mol Evol 1998; 47: 230-134.
34. Veena, Reddy VS, Sopory SK. Glyoxalase I from *Brassica juncea*: molecular cloning, regulation and its over-expression confer tolerance in transgenic tobacco under stress. Plant J 1999; 17: 385-395.
35. Skipsey M, Andrews CJ, Townson JK, Jepson I, Edwards R. Cloning and characterization of glyoxalase I from soybean. Arch Biochem Biophys 2000; 374: 261-268.
36. Johansen KS, Svendsen II, Rasmussen SK. Purification and cloning of the two domain glyoxalase I from wheat bran. Plant Sci 2000; 155: 11-20.
37. Usui Y, Nakase M, Hotta H, et al. A 33-kDa allergen from rice (*Oryza sativa* L. *Japonica*) cDNA: cloning, expression, and identification as a novel glyoxalase I. J Biol Chem 2001; 276: 11376-11381.
38. Bernstein FC, Koetzle TF, Williams GJ, et al. The protein data bank: a computer-based archival file for macromolecular structures. J Mol Biol 1977; 112: 535-542.
39. Cameron AD, Olin B, Ridderstrom M, Mannervik B, Jones TA. Crystal structure of human glyoxalase I - evidence for gene duplication and 3D domain swapping. EMBO J 1997; 16: 3386-3395.
40. Cameron AD, Ridderstrom M, Olin B, Kavarana MJ, Creighton DJ, Mannervik B. Reaction mechanism of glyoxalase I explored by an X-ray crystallographic analysis of the human enzyme in complex with a transition state analogue. Biochemistry 1999; 38: 13480-13490.
41. He MM, Clugston SL, Honek JF, Matthews BW. Determination of the structure of *Escherichia coli* glyoxalase I suggests a structural basis for differential metal activation. Biochemistry 2000; 39: 8719-8727.
42. Deswal R, Sopory SK. Purification and partial characterization of glyoxalase I from a higher plant *B. juncea*. FEBS Lett 1991; 282: 277-280.
43. Atlante A, deBari L, Valenti D, Pizzuto R, Paventi G, Passarella S. Transport and metabolism of D-lactate in Jerusalem artichoke mitochondria. Biochim Biophys Acta 2005; 1708: 13-22.
44. Norton SJ, Principato GB, Talesa V, Lupattelli M, Rosi G. Glyoxalase II from *Zea mays*: properties and inhibition study of the enzyme purified by use of a new affinity ligand. Enzyme 1989; 42: 189-196.
45. Maiti MK, Krishnasamy S, Owen HA, Makaroff CA. Molecular characterization of glyoxalase II from *Arabidopsis thaliana*. Plant Mol Biol 1997; 35: 471-481.
46. Saxena M, Bisht R, Roy SD, Sopory SK, Bhalla-Sarin N. Cloning and characterization of a mitochondrial glyoxalase II from *Brassica juncea* that is upregulated by NaCl, Zn, and ABA. Biochem Biophys Res Commun 2005; 336: 813-819.

Authenticated | xajibim@mail.ru
Download Date | 6/16/12 12:10 AM

47. Ridderstrom M, Mannervik B. Molecular cloning and characterization of the thiolesterase glyoxalase II from *Arabidopsis thaliana*. Biochem J 1997; 322: 449-454.
48. Marasinghe GP, Sander IM, Bennett B, et al. Structural studies on a mitochondrial glyoxalase II. J Biol Chem 2005; 280: 40668-40675.
49. Wenzel NF, Carenbauer AL, Pfiester MP, Schilling O, Meyer-Klaucke W, Makaroff CA. Crowder MW. The binding of iron and zinc to glyoxalase II occurs exclusively as di-metal centers and is unique within the metallo-beta-lactamase family. J Biol Inorg Chem 2004; 9: 429-438.
50. Zang TM, Hollman DA, Crawford PA, Crowder MW, Makaroff CA. *Arabidopsis* glyoxalase II contains a zinc/iron binuclear metal center that is essential for substrate binding and catalysis. J Biol Chem 2000; 276: 4788-4795.
51. Yang KW, Sobieski DN, Carenbauer AL, Crawford PA, Makaroff CA. Crowder MW. Explaining the inhibition of glyoxalase II by 9-fluorenyl-methoxycarbonyl-protected glutathione derivatives. Arch Biochem Biophys 2003; 414: 271-278.
52. Vander Jagt DL, Robinson B, Taylor KK, Hunsaker LA. Reduction of trioses by NADPH-dependent aldoketo-reductases. J Biol Chem 1992; 267: 4364-4369.
53. Oberschall A, Deak M, Torok K, et al. A novel aldose/aldehyde reductase protects transgenic plants against lipid peroxidation under chemical and drought stresses. Plant J 2000; 24: 437-446.
54. Hideg E, Nagy T, Oberschall A, Dudits D, Vass I. Detoxification function of aldose/aldehyde reductase during drought and ultraviolet-B (280-320) stresses. Plant Cell Environ 2003; 26: 513-522.
55. Misra K, Banerjee AB, Ray S, Ray M. Glyoxalase III from *Escherichia coli*: a single novel enzyme for the conversion of methylglyoxal into D-lactate without reduced glutathione. Biochem J 1995; 305: 999-1003.
56. Kersten PJ, Kirk TK. Involvement of a new enzyme, glyoxal oxidase, in extracellular H_2O_2 production by *Phanerochaete chrysosporium*. J Bacteriol 1987; 169: 2195-2201.
57. Baggetto LG, Lehninger AL. Isolated tumoral pyruvate dehydrogenase can synthesize acetone which inhibits pyruvate oxidation as well as other aldehydes. Biochem Biophys Res Commun 1987; 145: 153-159.
58. Ramaswamy O, Guha-Mukherjee S, Sopory SK. Presence of glyoxalase I in pea. Biochem Int 1983; 7: 307-318.
59. Ramaswamy O, Guha-Mukherjee S, Sopory SK. Correlation of glyoxalase I activity with cell proliferation in *Datura* callus culture. Plant Cell Rep 1984; 3: 121-124.
60. Sethi U, Basu A, Guha-Mukherjee S. Control of cell proliferation and differentiation by regulating polyamine biosynthesis in cultures of *Brassica* and its correlation with glyoxalase I activity. Plant Sci 1988; 56: 167-175.
61. Khan MM, Jan A, Karibe H, Komatsu S. Identification of phosphoproteins regulated by gibberellin in rice leaf sheath. Plant Mol Biol 2005; 58: 27-40.
62. Chitteti BR, Peng Z. Proteome and phosphoproteome differential expression

Authenticated | xajibim@mail.ru
Download Date | 6/16/12 12:10 AM

under salinity stress in rice (*Oryza sativa*) roots. J Proteome Res 2007; 6: 1718-1727.
63. Shin R, Alvarez S, Burch AY, Jez JM, Schachtman DP. Phosphoproteomic identification of targets of the *Arabidopsis* sucrose nonfermenting-like kinase SnRK2.8 reveals a connection to metabolic processes. Proc Natl Acad Sci USA 2007; 104: 6460-6465.
64. Bagga S, Das R, Sopory SK. Inhibition of cell proliferation and glyoxalase I activity by calmodulin inhibitors and lithium in *Brassica oleracea*. J Plant Physiol 1987; 129: 149-153.
65. Snedden WA, Fromm H. Calmodulin, calmodulin-related proteins and plant responses to the environment. Trends Plant Sci 1998; 3: 299-304.
66. Deswal R, Sopory SK. Glyoxalase I from *B. juncea* is calmodulin stimulated. Biochim Biophys Acta 1999; 1450: 460-467.
67. Jain M, Choudhary D, Kale RK, Bhalla-Sarin N. Salt- and glyphosate-induced increase in glyoxalase I activity in cell lines of groundnut (*Arachis hypogaea*). Physiol Plant 2002; 114: 499-505.
68. Singla-Pareek SL, Yadav SK, Pareek A, Reddy MK, Sopory SK. Transgenic tobacco overexpressing glyoxalase pathway enzymes grow and set viable seeds in zinc spiked soils. Plant Physiol 2006; 140: 613-623.
69. Singla-Pareek SL, Yadav SK, Pareek A, Reddy MK, Sopory SK. Enhancing salt tolerance in a crop plant by overexpression of glyoxalase II. Transgenic Res 2007; Epub ahead of print 11248-007-9082-2.
70. Yadav SK, Singla-Pareek SL, Ray M, Reddy MK, Sopory SK. Methylglyoxal levels in plants under salinity stress are dependent on glyoxalase I and glutathione. Biochem Biophys Res Commun 2005; 337: 61-67.
71. Yadav SK, Singla-Pareek SL, Reddy MK, Sopory SK. Methylglyoxal detoxification by glyoxalase system: a survival strategy during environmental stresses. Physiol Mol Biol Plants 2005; 11: 1-11.
72. Chaplen FW, Fahl WE, Cameron DC. Evidence of high levels of methylglyoxal in cultured Chinese hamster ovary cells. Proc Natl Acad Sci USA 1998; 95: 5533-5538.
73. Martins AMTBS, Cordeiro CAA, Freire AMJP. In situ analysis of methylglyoxal metabolism in *Saccharomyces cerevisiae*. FEBS Lett 2001; 499: 41-44.
74. Yadav SK, Singla-Pareek SL, Reddy MK, Sopory SK. Transgenic tobacco plants overexpressing glyoxalase enzymes resist an increase in methylglyoxal and maintain higher reduced glutathione levels under salinity stress. FEBS Lett 2005; 579: 6265-6271.
75. Sgherri CLM, Navari-Izzo F. Antioxidative enzymes in wheat subjected to increasing water deficit and rewatering. J Plant Physiol 2000; 157: 273-279.
76. Foyer CH, Lopez-Delgado H, Dat JF, Scott IM. Hydrogen peroxide and glutathione associated mechanisms of acclimatory stress tolerance and signaling. Physiol Plant 1997; 100: 241-254.
77. Ahsan N, Lee DG, Lee SH, et al. Excess copper induced physiological and proteomic changes in germinating rice seeds. Chemosphere 2007; 67: 1182-1193.

Authenticated | xajibim@mail.ru
Download Date | 6/16/12 12:10 AM