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Amino Acids

Analysis of methionine oxides and nitrogen-transporting amino acids in chilled and acclimated maize seedlings*

A. D. Simonović and **M. D. Anderson**

Department of Biological Sciences, North Dakota State University, Fargo, ND, U.S.A.

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Summary. In maize seedlings, chilling causes a reduction of glutamine synthetase (GS) activity, while acclimation protects GS (manuscript submitted). Since ROS can oxidize both protein-bound and free Met to methionine sulfoxide (MSO) and further to methionine sulfone (MSO2, a GS inhibitor), it was hypothesized that the chilling-induced oxidative stress may cause accumulation of MSO and MSO2, thus contributing to the inactivation of GS. MSO2 preferentially inhibited the chloroplastic isoform, GS2. HPLC analysis of polar amino acids from coleoptiles + leaves, mesocotyls and roots of control, chilled, acclimated, acclimated and chilled and chilled and rewarmed plants revealed that free MSO and MSO2 do not accumulate after low temperature treatments. Nevertheless, acclimation significantly increased the expression of putative protein methionine sulfoxide reductase (PMSR), especially in mesocotyls. Different low temperature treatments caused complex changes in the profiles of N-transporting amino acids, Asp, Glu, Asn and Gln.

Keywords: Glutamine synthetase – Methionine oxides – N-transporting amino acids – Chilling – Acclimation – PMSR

Abbreviations: C + l, coleoptiles + leaves; d, day; GS, glutamine synthetase; GS1, cytosolic isoform of glutamine synthetase; GS2, plastidic isoform of glutamine synthetase; MSN, L-methionine-S-sulfoximine; MSO, L-methionine sulfoxide; MSO2, L-methionine sulfone; PMSR, protein methionine sulfoxide reductase; ROS, reactive oxygen species

Introduction

Glutamine synthetase (GS, E.C. 6.3.1.2) plays a central role in nitrogen metabolism by catalyzing the assimilation of ammonia into glutamine. Plants have cytosolic GS1 isoforms, predominant in nonphotosynthetic tissues, and plastidic isoform GS2, the major form in green leaves (Hirel and Gadal, 1980, 1982; Lam et al., 1996). The maize genome contains the largest GS gene family described in plants to date, comprised of six single-copy nuclear genes, one for GS2 (Snustad et al., 1988), and five for cytosolic isoforms, named GS1-1 through GS1-5 (Sakakibara et al., 1992; Li et al., 1993).

We have shown that in Pioneer G50, a chilling-sensitive and acclimation-responsive maize inbred, chilling causes a reduction of GS activity, while acclimation protects GS (manuscript submitted). Chilling (7 days at 4 °C) induces accumulation of H_2O_2, causing severe oxidative stress, which seedlings cannot survive unless acclimated at 14 °C (Prasad et al., 1994a, b; Anderson et al., 1995). The acclimation response includes induction of mitochondrial catalase, several peroxidases and a change of the glutathione reductase isozyme profile (Prasad et al., 1994a, 1995; Anderson et al., 1994, 1995). The chilling-induced oxidative stress probably results in oxidative degradation of GS, while the protective effect of acclimation can be explained by accumulation of antioxidative enzymes. We hypothesize that an additional mechanism of GS inactivation at low temperatures involves accumulation of free MSO and MSO2 in plants, and that accumulated MSO2 acts as a GS inhibitor. Namely, methionine derivatives L-methionine-S-sulfoximine (MSN) and MSO2 are prominent GS inhibitors, which share structural homology with the tetrahedral intermediate formed in the GS active site, thus serving as transition state analogs (Liaw and Eisenberg, 1994; Eisenberg et al., 2000). The side chains of both free and protein-bound Met can be oxidized by a number of different reactive oxygen species (ROS), including $O_2^{\bullet -}$, H_2O_2, $OH^{\bullet}$ and peroxynitrite ($ONOO^-$), as well as by other oxidants such as periodate (IO_4^-), to MSO, which,

* This paper is dedicated to the memory of Dr. Radomir Konjević, professor of plant physiology, who passed away in July 2006, on the eve of his 60th birthday.

in the presence of a strong oxidant, can be further oxidized to MSO2 (Yamasaki et al., 1982; Stadtman, 1993; Hoshi and Heinemann, 2001). The aim of this work is to determine whether Met oxides accumulate during the chilling stress to the levels that might inhibit GS.

The oxidation of Met to MSO is biologically reversible, by the action of peptide methionine sulfoxide reductase (PMSR), but the oxidation of MSO to MSO2 is irreversible (Hoshi and Heinemann, 2001; Romero-Angulo, 2005). Most organisms have two (families of) PMSR enzymes that can act upon both free and protein-bound MSO: PMSRA (or MsrA), specific for L-Met-S-SO, and structurally unrelated PMSRB (or MsrB), which reduces L-Met-R-SO (Grimaud et al., 2001; Romero-Angulo, 2005). The expression of PMSR was analyzed, to see whether it is induced during the acclimation, along with other antioxidative enzymes.

It is expected that the observed inactivation of GS by chilling would cause an alteration of N-transporting amino acid profile in the stressed seedlings. The N-transporting amino acids, Gln, Glu, Asp and Asn, are the major free amino acids in plants and are first synthesized during N assimilation (Corruzi and Last, 2000). The study of the effect of different low temperature treatments on the amount of Gln, Glu, Asp and Asn in different seedling organs is another objective of this work.

Materials and methods

Chilling and acclimation treatments

Maize inbred G50 was grown in pots with Sunshine Germinating Mix #3 on continuous light (combined fluorescent and incandescent white light, 100 μmol m^{-2} s^{-1}) at 27 °C for 7 d for the inhibition studies, or in darkness at 27 °C for 4 d (control, G4), followed by different low temperature treatments, for the amino acid analyses. The temperature treatments included: acclimation (A, 3 d at 14 °C), chilling (C, 7 d at 4 °C), chilling and rewarming (CR, 7 d at 4 °C followed by 1 d at 27 °C) and acclimation followed by chilling (AC, 3 d at 14 °C, followed by 7 d at 4 °C). Since maize grows very little during acclimation and not at all during chilling, then A, C, and even 14 d old AC plants can be considered the same developmental stage as G4 controls (Anderson et al., 1994). The tissue samples of coleoptiles and leaves (c + l), mesocotyls and roots were frozen in liquid nitrogen and stored at −80 °C until protein, amino acid or RNA isolation.

Protein extraction, GS assay and inhibition studies

Soluble proteins were extracted from green, 7 d old c + l samples (which have high and approximately equal GS1 and GS2 activities), separated by native PAGE and assayed for GS activity with 10 mM substrates, ATP, Glu and NH_4Cl, as described in Simonović et al. (2004). To test whether the inhibition was irreversible, 1-lane gel strips with separated proteins were placed in inhibitor solutions, incubated 20 min at RT, washed 3 times with water for 10 min, and then assayed for GS activity. Alternatively, the strips were assayed in a buffer containing both substrates (10 mM each) and 0–20 mM inhibitors. The gels with activity bands were scanned, and the optical density was quantified using ImageJ software (ver. 1. 32J, National Institute of Health, USA). To minimize gel-to-gel differences, the background was subtracted prior to analysis. All data are average values of three independent repeats.

Amino acid extraction and preparation of the samples for HPLC

Frozen samples of etiolated c + l, mesocotyls and roots of G4, A, C, AC, and CR seedlings, in 3 replicates, were used for amino acid extraction. One gram of tissue was grinded in liquid nitrogen, homogenized in 4 ml methanol and spun 5 min at 14,000 g. The extracts were mixed with 2 ml chloroform and 3 ml water. The aqueous phase was re-extracted with chloroform, evaporated to dryness, and stored at −20 °C until use. The dry samples were dissolved in 100 μl water and purified by solid phase extraction using Bakerbond SPE Octadecyl Disposable Extraction Columns (500 mg, 6 ml, J. T. Baker). Polar amino acids were eluted from the columns with 0.9 ml water. C + l and mesocotyl samples were diluted 50×, while the root samples were diluted 20× with water, 100 μl aliquots were oxidized with 10 μl 200 mM sodium periodate for 20 min at RT, and the pH was adjusted with 10 μl 2 M borate buffer pH 9.9. Amino acids were derivatized by addition of 80 μl 10 mM FMOC-Cl (9-fluorenylmethyl chloroformate, Bachem) in dehydrated acetonitrile. The excess reagent was removed by heptane extraction. The prepared samples were filtered through a 0.2 μm filter and 10 μl were injected on the column. Solutions of standard amino acids (all purchased from Sigma) were treated the same way as the samples.

Reverse-phase HPLC analysis of polar amino acids

HPLC analysis was performed using a Beckman System Gold Programmable Solvent Module 126. Derivatized amino acids were separated on a Mightysil RP-18 GP Aqua column (150 × 4.6 mm I.D., 5 μm particle size, Kanto Corporation) kept at 38 °C, at constant flow rate of 1.4 ml/min. 25 mM citrate buffer pH 6.4 (A) and 90% methanol (B) were applied in the following elution gradient: 43% B (initial), 47% B (1st min; duration of the change 15 min), 90% B (18th min; duration 5 min), 43% B (30th min, duration 3 min), end of run (40th min). The fluorescence was detected using Shimadzu Fluorescence HPLC Monitor RF 530, with excitation and emission wavelengths set at 265 and 315 nm, respectively. The signal was integrated using Hewlett Packard HP 3396A Integrator.

Analysis of total amino-nitrogen by ninhydrin

Total amino nitrogen was determined by the ninhydrin reaction. SPE-purified samples (undiluted, 20 μl) were mixed with 50 μl of ninhydrin reagent (350 mg of ninhydrin in 100 ml ethanol) and boiled for 4 min, resulting in development of dark blue color. Cooled samples were diluted with 930 μl ethanol and the absorbance was measured at 570 nm. The concentration of amino-nitrogen was calculated using a glycine-based standard curve.

RNA extraction and quantitative RT-PCR analysis

Total RNA was extracted from organs of seedlings acclimated for different time, using Trizol Reagent (Invitrogen). The RNA samples (2 μg) were treated with RQ1 DNAse (Promega) and reverse transcribed using Omniscript Reverse Transcription kit (Qiagen) with Anchored Oligo$(dT)_{23}$ primers (Sigma, 1 μM final concentration) and 10 U/reaction RNAse inhibitor (Promega). SYBR Green real-time PCR assays were set by mixing 2 μl cDNA templates (corresponding to 200 ng total RNA) with 12.5 μl 2× QuantiTect SYBR Green PCR Master Mix (Qiagen), 2 μl combined forward and reverse primers (final 0.3 μm each) and PCR-grade water to 25, μl in 96-well optical plates. A 101 bp sequence of putative maize

PMSR represented by ESTs AY108550 and CK327555 was amplified using the forward primer TTGGCAACCAGTATAGATCTGTCATC and the reverse primer GCTGCTACGGTCTTTAGCTTGTTC. The specificity of the primers was checked by "blasting" against the maize database MaizeGDB (http://www.maizegdb.org/), and confirmed by electrophoretic sizing and by melting curve analysis. Real-time PCR was done using a Stratagene Mx3000P thermal cycler, with the following cycling program: initial activation step 95 °C/15 min, denaturation 94 °C/15 s, annealing 55 °C/30 s and extension 72 °C/30 s, for 45 cycles. The relative expression was calculated from the amplification efficiencies using Liu and Saint's method (2002), with actin as a housekeeping gene (forward primer: TCTGCTGAACGCGAAATTGT; reverse: ACAGATGAGCTG CTCTTGGCA).

Results and discussion

Inhibition of GS1 and GS2 by methionine derivatives

The effect of Met and its derivatives, MSO, MSO2 and MSN on GS1 and GS2 activities was tested. Neither Met nor MSO showed any inhibitory effect, while MSN (Fig. 1) and MSO2 (Fig. 2) were inhibitory. Because the GS assay is gel-based (Simonović et al., 2004), it allows experiments with preincubation of the gel strips with the enzyme in inhibitors, followed by washing the gels and assaying the remaining activity. This can reveal whether the inhibition is reversible (if the inhibitor can be washed out without affecting GS) or irreversible (if the inhibition remains after washing). MSN equally inhibits both GS1 and GS2, to essentially the same extent whether applied as preincubation or simultaneously with the substrates (Fig. 1). Tomoyuki et al. (1988) also found that MSN inhibits both isoforms in maize. When MSN is applied in the presence of ATP and Mn^{2+} or Mg^{2+}, its Nε nitrogen is phosphorylated by GS to give methionine sulfoximine phosphate and ADP (Ronzio and Meister, 1968). In *E. coli* GS, phosphorylated MSN, ADP and the two metal ions bind synergistically and lock on the enzyme subunit as inactivation complex, which strengthens the subunit-subunit interactions (Hunt and Ginsburg, 1980; Maurizi and Ginsburg, 1982). Therefore, it has been postulated that MSN phosphorylation is essential for the irreversible inhibition of GS, and MSN is considered an ATP-dependent inactivator (Eisenberg et al., 2000). The current results, however, show that MSN can inhibit maize GS irreversibly even in an ATP-independent manner. This difference in inhibition requirements may be due to the difference between bacterial and plant GS.

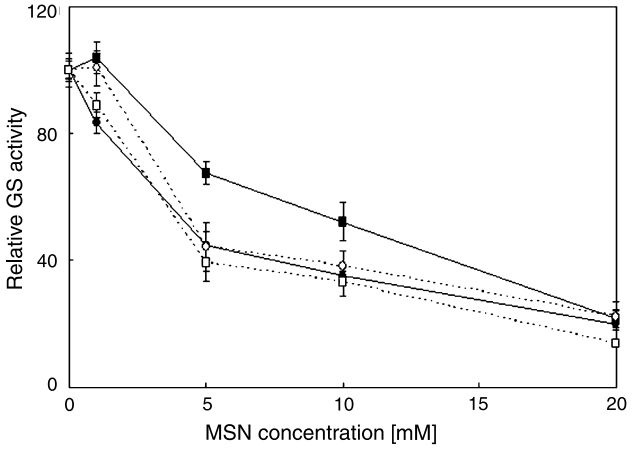

Fig. 1. Inhibition of GS1 and GS2 by MSN. Soluble proteins (50 µg/lane) were separated by native PAGE, and 1-lane gel strips were assayed for GS activity in the presence of increasing concentrations of MSN and 10 mM substrates applied simultaneously (full line, ● – GS2, ■ – GS1) or preincubated in increasing concentrations of MSN, washed, and then assayed for GS activity (dashed line, ○ – GS2, □ – GS1). The GS activity is presented relative to the control without inhibitor

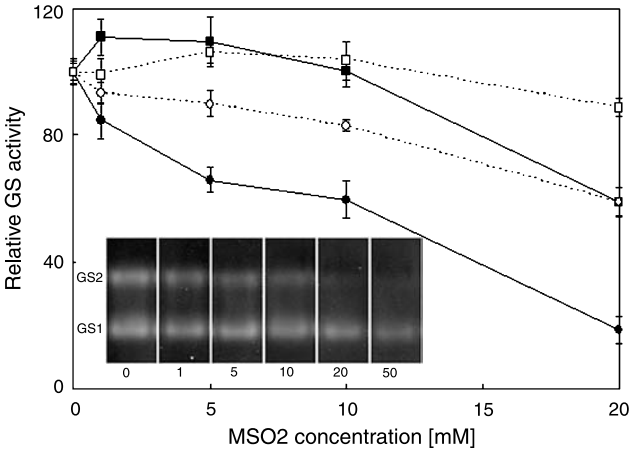

Fig. 2. Sensitivity of GS1 and GS2 to MSO2. Soluble proteins (50 µg/lane) were separated by native PAGE, and 1-lane gel strips were assayed for GS activity in the presence of increasing MSO2 concentrations and 10 mM substrates applied simultaneously (full line, ● – GS2, ■ – GS1 and the insert), or preincubated in increasing concentrations of MSO2, washed, and then assayed for GS activity (dashed line, ○ – GS2, □ – GS1). The GS activity is presented relative to the control

Unlike MSN, MSO2 was virtually ineffective when applied as a pretreatment and washed, and only slightly inhibited GS2 at 20 mM concentration, which may be due to some residual MSO2 that remained in the gel (Fig. 2). This means either that MSO2 binding to GS is reversible, or that ATP is required for the inhibition. MSO2 applied together with the substrates inhibited primarily GS2, while significant proportion of GS1 remained active even after incubation with 50 mM MSO2 (Fig. 2, insert). Thus, even if free MSO2 is generated during the chilling, it would have to accumulate to the levels comparable to substrate, Glu, in order to have physiologically significant effect.

Sample preparation and separation of standard amino acids by HPLC

To analyze the content of methionine oxides and N-transporting amino acids after low temperature treatments, a HPLC protocol has been developed, based on Or-Rashid et al's (2000) method. Satisfactory separation of all amino acids of interest, as well as Arg, Gly and Ser that were present in SPE-purified samples has been accomplished, with exception of MSO and Ser, so an approach based on Ser oxidation has been adopted (Fig. 3). Ser is one of the amino acids most sensitive to periodate oxidation (Clamp and Hough, 1965), so it was quantitatively eliminated under mild oxidation conditions applied, while none of the other analytes showed any measurable decline (Fig. 3). The treatment of Met with periodate yields MSO, which is further oxidized to MSO2 (Yamasaki et al., 1982). HPLC analysis of Met and MSO oxidation by periodate revealed that Met is, indeed, rapidly and quantitatively converted to MSO (data not shown), but MSO is not oxidized to MSO2 (Fig. 3). If Met was present in the samples, it would interfere with MSO quantification, but is was quantitatively retained by SPE columns (data not shown).

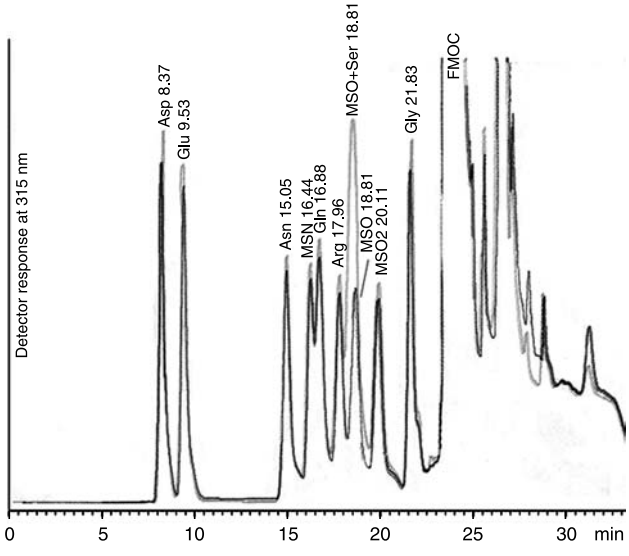

Fig. 3. Chromatogram of standard amino acids. Mixture of 10 amino acids, 25 pmol each, was separated by HPLC (gray line). Oxidation of the same mixture with periodate quantitatively eliminates Ser, but does not affect other amino acids (overlaid black line). The retention times presented are average values from at least 11 (up to 18) runs, with standard errors ranging from 0.02 to 0.05 min (not shown)

Effect of chilling and acclimation on the profile of free polar amino acids and methionine oxides in different organs of etiolated seedlings

Chilling may cause an increase in electrolyte leakage and water soaking of the plant tissues (Saltveit and Moris, 1990) as well as water stress in maize (Melkonian et al., 2004), so expressing the amount of amino acids per unit of fresh weight (FW) would be biased. Table 1 shows that DW/FW ratio in chilled seedlings is dramatically increased in coleoptiles, but only slightly in mesocotyls; A and AC treatments also cause a small increase of DW/FW ratio in shoots. In roots, however, C and CR treatments cause a reduction of DW/FW ratio in comparison to the G4, A and AC treatments. Therefore, the amount of amino acids in tissue samples determined by HPLC is presented per unit of amino nitrogen, which correlates with the DW of the samples (Table 1), rather than per unit of FW.

The MSO peak was found in all c + 1 and root samples, irrespective of the temperature treatment (Figs. 4 and 6), while in mesocotyls, it was prominent in G4 and A samples, but absent in C and AC samples (Fig. 5). Only a trace amount of MSO2 was found in c + 1 samples (1.2–2.2 nmol/μmol amino N) and CR mesocotyl samples (0.27 nmol/μmol amino N). The identity of the MSO and

Table 1. The amount of amino N in different samples is expressed per g FW, as an average of 3 replications. Some of the tissue samples from the same pool of samples used for the ninhydrin and HPLC analyses were dried to calculate the DW/FW ratio, but there were not enough CR samples of coleoptiles and mesocotyls

	Coleoptiles + leaves		Mesocotyls		Roots	
	μmol amino N per g FW	mg DW per g FW	μmol amino N per g FW	mg DW per g FW	μmol amino N per g FW	mg DW per g FW
G4	8.9 ± 0.8	60 ± 4	2.6 ± 0.3	34 ± 2	7 ± 1	42 ± 2
A	18.4 ± 0.5	70 ± 4	2.8 ± 0.7	35 ± 2	6.5 ± 0.8	42 ± 2
C	33 ± 2	144 ± 4	4.0 ± 0.8	41 ± 2	4.9 ± 0.4	27 ± 2
CR	50 ± 8	–	4.3 ± 0.2	–	3.0 ± 0.7	23 ± 2
AC	28 ± 2	71 ± 5	3.2 ± 0.7	39 ± 2	9 ± 1	34 ± 2

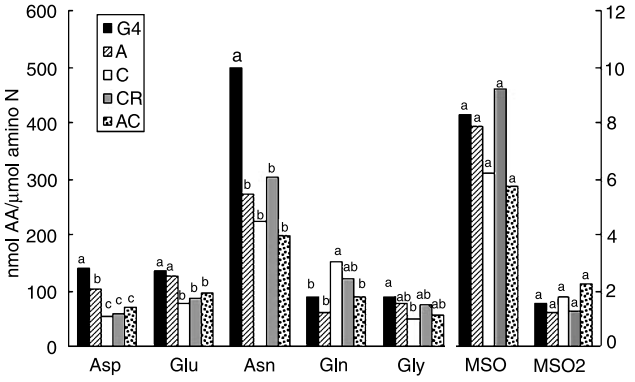

Fig. 4. Profile of polar amino acids in etiolated coleoptiles + leaves after different temperature treatments. For each amino acid, the results represent the mean of three replicates. Means with the same letter above the values are not statistically different (P = 0.05). Note the different y-scale used for the major amino acids (left) and methionine oxides (right)

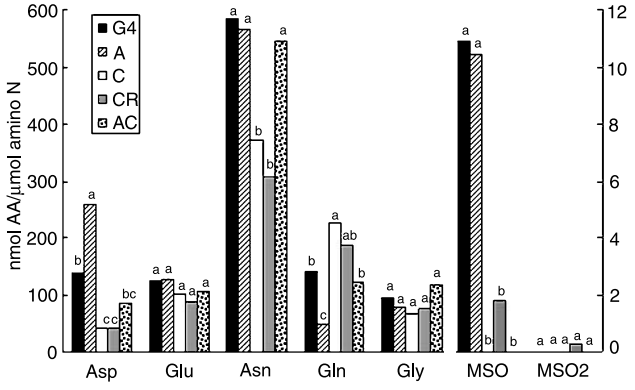

Fig. 5. Profile of polar amino acids in mesocotyls of etiolated seedlings after different temperature treatments

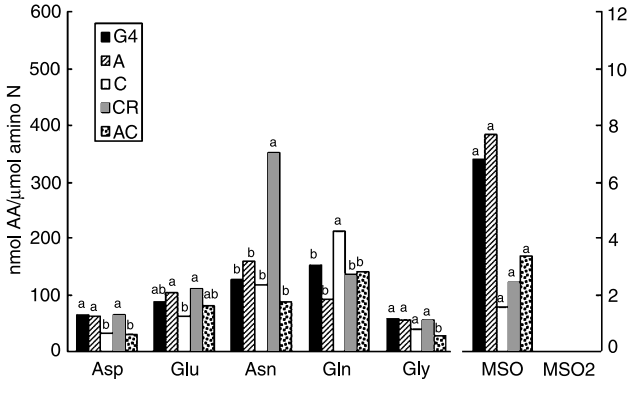

Fig. 6. Profile of polar amino acids in roots of etiolated seedlings after different temperature treatments

MSO2 peaks has not been confirmed by mass spectrometry or other means, but it can be concluded that these compounds do not accumulate during chilling. The amount of MSO2, where present, is ≈100 times lower than the substrate, Glu, so its inhibitory effect on GS2 would be negligible anyway, unless it accumulates locally. MSN was also considered in the analysis to see whether it could be generated nonenzymatically in stressed cells (Fig. 3). However, since MSN elutes between large Asn and Gln peaks, its detection was impossible using the current protocol.

Few papers describe the changes in N-transporting and other free amino acids in plants exposed to low temperatures, and these are controversial, inconclusive, and depend on the type of the treatment, plant species, ecotype/cultivar, plant organ and the analytical method used. The amounts of Asp and Glu decreased with chilling. Asp particularly decreased in C and CR mesocotyl samples, while Glu decreased somewhat in c + l or insignificantly in mesocotyls (Figs. 4 and 5). Asp was the only amino acid that accumulated in the response to acclimation, but only in mesocotyls (Fig. 5). Literature reports do not offer an explanation on the importance of these changes. In bluegrass, Asp and Glu decreased after acclimation at 2 °C, but increased over winter in the freezing-sensitive ecotype (Dionne et al., 2001). In wheat exposed to cold stress, Asp and Glu accumulated in crowns (Kaldy and Freyman, 1984), but in leaves Asp increased, while Glu decreased (Naidu et al., 1991). Meza-Basso et al. (1986) observed that Asp and Glu levels decreased in leaves of *Nothofagus dombeyi* upon exposure to 0 °C for 24 h.

Asn is the major amino acid in all seedling organs, which is expected for the dark-grown plants, because of its high N/C ratio (Corruzi and Last, 2000; Oliviera et al., 2001). In c + l, the amount of Asn significantly decreased upon exposure to any low temperature treatment, while in mesocotyls it decreased after C and CR treatments, but not if the seedlings were acclimated. In roots, however, the amount of Asn after CR was significantly higher than in other treatments, perhaps because CR impairs the xylem transport of Asn from roots to shoots. In overwintering crowns of annual bluegrass, Asn increased during subzero acclimation in a freezing-sensitive ecotype, while the changes of Asn during winter were complex (Dionne et al., 2001).

Since chilling inactivates and acclimation protects GS (manuscript submitted), it would be expected that the Gln level decrease after chilling and increase after acclimation, but exactly the opposite was found in all organs, particularly in mesocotyles. In all AC samples Gln was at the level of control. It is possible that chilling impairs other assimilatory enzymes that use Gln (such as GOGAT) more than it affects GS. Wheat seedlings exposed to cold stress also accumulate Gln (Naidu et al., 1991), as well

as a freezing-sensitive bluegrass ecotype during subzero acclimation, while in a freezing-tolerant ecotype, Gln decreased (Dionne et al., 2001). The changes in Gly levels after different treatments were minor, whereas Arg appeared as a small shoulder on the large Gln peak, which in some samples was not integrated (data not shown).

The described changes in the polar amino acid profiles after C and CR treatments probably reflect differential effects of chilling and/or oxidative stress on GS and other nitrogen assimilatory enzymes, as well as impairment of vascular transport through chilling-injured organs. All of these changes can be considered as pathological and not tolerance-related, because none of the tested analytes, except Asp in mesocotyls, increased in response to acclimation. Acclimation as a pretreatment to chilling, however, efficiently prevented large changes in the amino acid profile in all organs except Asp and Asn in c + 1 and Asp in mesocotyls, so that amino acid levels in AC samples were at or close to the control levels.

Induction of putative PMSR is a part of the acclimation response

Even though free Met oxides do not accumulate during chilling, it is possible that oxidation of Met residues in GS is related to its inactivation by chilling. Maize GS2 has four, while each of the GS1 isoforms have 7 Met residues per subunit, but It is not known if they are exposed and susceptible to oxidation as in *E. coli* GS (Levine et al., 1996, 1999). Recent analysis of the plant PMSR family, including a BLAST search of the GenBank using *Arabidopsis* PMSRA as a query, revealed a presence of PMSR-like proteins in *Brassica* sp., *Oryza sativa* and few other plants (Romero-Angulo, 2005), but no maize sequences had been identified at that time. However, it seems that maize EST AY108550 qualifies as a putative PMSR, because it shares 41.6 and 43.5% nucleotide sequence identity with *Arabidopsis* PMSRs NM_201754 and NM_127359, and 58.9% identity with rice putative PMSR (BE530943). A part of AY108550 is identical with shorter EST, CK327555, isolated from maize sperm cell cDNA library, and annotated as ''sequence similar to PMSR'' (Engel et al., 2003).

The expression of putative PMSR has been analyzed by real-time PCR during the 3-day course of acclimation (Fig. 7). A two-fold induction of PMSR was observed in roots after 6 h into the acclimation, but the transcript level did not change any further. The expression in c + 1 significantly increased, with a maximum of 8-fold after 2 days of acclimation, followed by a decline. The strongest induction of nearly 12 fold by the end of the experiment was observed in mesocotyls. The onset of acclimation in G50 seedlings is marked by a rapid increase of H_2O_2 in mesocotyls, and this mild oxidative stress is thought to induce ROS-scavenging enzymes, such as catalase 3 (Prasad et al., 1994a); PMSR is also known to be induced by oxidative stress, in *Arabidopsis* (Romero et al., 2004; Dos Santos et al., 2005). The PMSR induction is considerably delayed relative to the CAT3 induction, which reaches maximum after 18 h of acclimation (Prasad et al., 1994a), but this relates to their roles: while CAT3 is the first line defense against the mitochondria-generated peroxide (Anderson et al., 1994; Prasad et al., 1994a, b), PMSR is the last-chance defense (Levine et al., 1999).

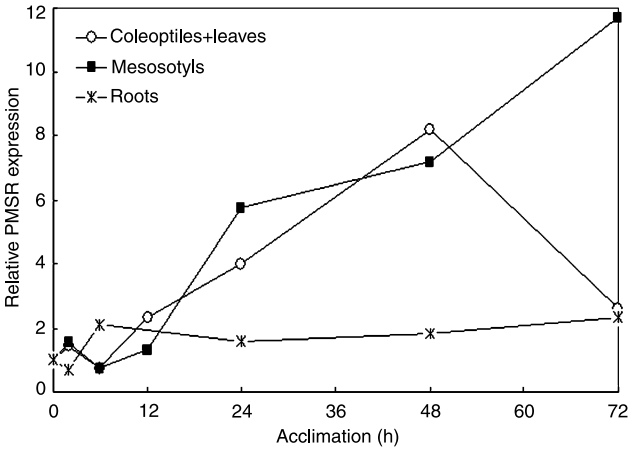

Fig. 7. Effect of acclimation on the expression of putative PMSR in etiolated seedlings. The expression of EST AY108550 (CK327555) in different seedling organs over the course of acclimation is presented relative to its expression in the corresponding nonacclimated (G4) samples

It can be concluded that free Met oxides do not accumulate during chilling and are likely irrelevant as physiological GS inhibitors. Further research is needed to evaluate the importance of oxidation and reduction of Met residues in maize GS isoforms, but the induction of putative PMSR is an important part of the acclimation response. Chilling causes a disturbance in N assimilation, resulting in a complex change of the N-transporting amino acids profile. Acclimation to chilling does not involve accumulation of analyzed amino acids, but prevents the chilling damage to a certain degree.

Acknowledgements

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Authors' address: Ana D. Simonović, Department of Plant Physiology, Institute for Biological Research "Siniša Stanković", Bulevar despota Stefana 142, 11000 Belgrade, Serbia,
Fax: +381 11 2078-404, E-mail: ana.simonovic@ibiss.bg.ac.yu