Hg and Cd induced changes in proline content and activities of proline biosynthesizing enzymes in Phaseolus aureus and Triticum aestivum

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Abstract

The effect of mercury and cadmium, in the form of HgCl₂ and CdCl₂ respectively, on proline accumulation and two key proline biosynthesizing enzymes, Δ¹-pyrroline-5-carboxylate synthetase (P5CS) and Δ¹-pyrroline-5-carboxylate reductase (P5CR), was investigated in Phaseolus aureus Roxb. and Triticum aestivum L. The 5-d-old seedlings were exposed to 0.05, 0.1, 0.2 or 0.4 mM concentrations of the metals in Hoagland solution for 12 and 36 h. T. aestivum exhibited considerably greater accumulation of proline than P. aureus in response to the metal treatment. Among the two metals, Hg induced greater accumulation of proline than Cd. The activity of P5CS increased significantly in response to the metal treatment, particularly in T. aestivum in which the activity of the enzyme in the control was much higher than that in P. aureus. The activity of P5CR on the other hand mostly decreased in response to the metal treatment. The study indicated a strong dependence of the metal induced proline accumulation on the constitutive P5CS content of the plants.

Additional key words: bean, heavy metals, Δ¹-pyrroline-5-carboxylate synthetase, Δ¹-pyrroline-5-carboxylate reductase, wheat.

Introduction

Free proline has been reported to accumulate in plants in response to a wide range of environmental stresses (for review see Hare and Cress 1997). The accumulation is also widely spread in plants in response to heavy metals (Alia and Saradhi 1991, 1993, Costa and Morel 1994, Schat et al. 1997). The compound has been attributed to a variety of functions of which its function as an osmoprotectant under drought and salinity stress has been widely advocated (Yoshita et al. 1997, and the references therein). The functional significance of proline accumulation in plants under heavy metal stress is, however, yet to be elucidated well. While a few studies (Alia and Saradhi 1991, 1993) in this regard suggest the synthesis of proline and its subsequent accumulation to be a process of non-toxic sink for the excess reductant (NADPH/NADH) accumulating as a result of disturbances in the metabolic processes upon exposure of the plants to heavy metals, other studies (Costa and Morel 1994, Schat et al. 1997) suggest the accumulation of the compound to be simply a consequence of development of metal-induced water deficit without having any role to play in over-coming the metal-induced toxicity. Hence, understanding the reason of accumulation of the compound in plants in response to heavy metal treatment needed further investigation.

Few studies were carried out to see the species-specific response of plants to heavy metals, and also metal specific response of a plant species, in terms of accumulation of proline. Reports on the effect of heavy metals on the activity of proline biosynthesizing enzymes were also scant. Hence, the present work was designed to study changes in the dry matter content, the concentration of proline and the activities of two crucial enzymes of the proline biosynthesis pathway from glutamate, Δ¹-pyrroline-5-carboxylate synthetase (P5CS) and Δ¹-pyrroline-5-carboxylate reductase (P5CR), in Phaseolus aureus Roxb. (a dicot) and Triticum aestivum L. (a monocot), in response to mercury and cadmium.

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Abbreviations: P5CR - Δ¹-pyrroline-5-carboxylate reductase; P5CS - Δ¹-pyrroline-5-carboxylate synthetase

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Materials and methods

The seeds of *P. aureus* and *T. aestivum* were soaked in distilled water overnight and then germinated and grown on a nylon net over 100 cm² Hoagland solution in a growth chamber (temperature of 25 ± 2 °C, irradiance of 200 μmol m⁻² s⁻¹, and 12-h photoperiod).

Analytical grade mercuric chloride (HgCl₂) and cadmium chloride (CdCl₂) were used for the treatment. In our earlier study (Shaw and Rout 1998) it was observed that Cd at concentration 30 μM or greater was lethal to the seedlings of *P. aureus* when applied after the 5th day of germination, but not when applied on or before the 5th day. Hg had no such age-dependent effect, and the seedlings survived irrespective of whether treated at an early or late stage of germination. An initial test on the seedlings of *T. aestivum* revealed no lethal effect of either Cd or Hg applied (in concentration as high as 1 M) on any day from the 4th to 9th day of germination. Based on the above information, the seedlings of the two plant species were exposed to 4 sub-lethal concentrations, 0.05, 0.1, 0.2 and 0.4 mM, of either Hg or Cd on the 5th day of germination. The seedlings not treated with the metals served as the control.

The seedlings from both treated and untreated sets were harvested after 12 and 36 h of exposure. The portions above the cotyledons in case of *P. aureus* and above the seed in case of *T. aestivum* were considered for the dry matter determination. The analysis of proline and the enzymes involved in its synthesis were done only in the leaves considering them as the site of maximum metabolic activities. The dry mass of the seedlings was obtained after drying them properly for 24 h in an oven at 140 °C. The leaves meant for proline and enzyme analysis were frozen in liquid N₂ and stored at -70 °C.

Proline contents of the leaves were determined following the procedure of Bates et al. (1973). For the enzyme analysis the leaves were homogenized in an extraction buffer (pH 7.5) containing 100 mM Tris-HCl, 10 mM MgCl₂, 1 mM EDTA, 10 mM β-mercaptoethanol, 4 mM dithiothreitol, 2 mM PMSF (phenylmethylsulfonyl fluoride) and 2 % polyvinylpolypyrrolidone (Chilson et al. 1992) in pre-chilled mortars and pestles in a cold room. The homogenates were centrifuged twice at 4 °C for 20 min at 20000 g. The activity of PCSR was assayed as proline-dependent reduction of NAD⁺ (the reverse reaction) following Chilson et al. (1992). The reaction was carried out in a final volume of 1 cm³ sodium glycinate buffer (200 mM, pH 10.3) containing 20 mM proline, 15 mM NAD⁺ and the enzyme extract. The activity was determined at 25 °C by monitoring the formation of NADH at 340 nm on an spectrophotometer (DU-68, Beckman, Fullerton, USA), and expressed as unit (U) mg⁻¹(protein); 1 U is defined as the amount of the enzyme required to generate 1 μmol of NADH in 1 min.

The activity of PCSCS in the same enzyme extract was determined as γ-glutamyl kinase by monitoring the formation of γ-glutamyl hydroxamate (Hayzer and Leisinger 1980). The enzyme mixture in a final volume of 0.5 cm³ contained 50 mM Tris-HCl (pH 7.0), 50 mM L-glutamate, 20 mM MgCl₂, 100 mM hydroxamate-HCl, 10 mM ATP and the enzyme extract. After addition of the extract, the reaction mixture was incubated at 37 °C for 15 min. The reaction was stopped by adding 1 cm³ of the stop buffer (2.5 g FeCl₃ and 6 g trichloroacetic acid in a final volume of 100 cm³ of 2.5 M HCl). The precipitated proteins were removed by centrifugation, and the absorbance of the clear supernatant was read at 535 nm against a blank identical to the above but lacking ATP. The activity was expressed in U mg⁻¹(protein); 1 U represented the amount of the enzyme required to produce 1 μmol of γ-glutamyl hydroxamate in 1 min.

The protein in the enzyme extract was quantified by the Coomassie brilliant blue dye binding method of Bradford (1976).

The data presented are the means of ten determinations for the dry matter content, five analyses for the proline content and six analyses for the enzyme activity. The difference between the means was tested by Duncan's multiple range test (Bliss 1967).

Results

The dry matter contents of the seedlings of both *P. aureus* and *T. aestivum* were reduced significantly upon exposure for 36 h to both Cd and Hg (Table 1). However, the effect of different concentrations of the metals was not significantly different from each other. 12-h exposure of the seedlings to the metals did not result in any significant reduction in their dry mass when compared to control.

The constitutive (control) content of proline was more or less same in both *P. aureus* and *T. aestivum*. However, the two species differed greatly in the amount of proline accumulated upon exposure to the metals. *P. aureus* did not show any significant change in proline content upon exposure to either Hg or Cd for 12 h (Fig. 1A). Upon 36-h exposure also the proline content increased significantly only in response to 0.1 mM or higher Hg concentration. In contrast *T. aestivum* exhibited significant accumulation of proline upon exposure for even 12 h to Hg as well as to Cd (Fig. 1B). The accumulation increased even more upon exposure for 36 h. The increase in the content of