Cd induced changes in proline level and peroxidase activity in roots of rice seedlings

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Abstract

The effects of Cd on changes in proline level and peroxidase activity in roots of rice seedlings were investigated. $CdCl_2$ was effective in inhibiting root growth and in accumulating proline in roots. The inhibition of root growth by Cd is reversible. The reduction of root growth induced by Cd is closely associated with accumulation of proline in roots. External application of proline markedly inhibited root growth of rice seedlings in the absence of Cd. Ionically bound, but not soluble, peroxidase activity in roots was increased by $CdCl_2$. Proline treatment also resulted in an increase in ionically bound peroxidase activity in roots. The relationship between growth inhibition of roots induced by Cd and changes of proline level and peroxidase activity is discussed.

Abbreviations: POX – peroxidase.

1. Introduction

Proline has been shown to accumulate in tissue/organs of plants subjected to drought-, salt- or temperature stress and/or infected by some pathogens [1, 3, 15], exposed to gases such as sulphur dioxide, ammonia or nitrogen dioxide [2]. Recently, Saradhi and Saradhi [17] provided evidence to show that Cd was the strongest inducer, among 4 tested heavy metals, for proline accumulation. Cd-induced increase in peroxidase (POX) activities has previously been reported for soybean and bean leaves [9, 13].

There are reports that proline accumulation is correlated with growth inhibition induced by NaCl [5]. It is not known whether such correlation exists in Cd-stressed plants. Growth inhibition may result from cell wall tightening process related to formation of cross-linkages among cell wall polymers by POX [8]. The ionically bound fraction of POX, removable from homogenized tissues with high ionic strength buffers, has been generally accepted to represent cell wall activity, and several studies have reported an association between increase in ionically bound POX activity and the timing or location of cessation of cell expansion [10, 11, 14]. No information is available about the correlation between ionically bound POX activity and plant growth under Cd stress. The present investigation was designed to study the changes in proline level and POX activity in Cd-stressed roots of rice seedlings and their correlation with root growth.

2. Materials and methods

Rice (*Oryza sativa* L., cv. Taichung Native 1) seeds were sterilized with 2.5% sodium hypochlorite for 15 min and washed extensively with distilled water. These seeds were then germinated in Petri dish (20 cm) containing distilled water at 37 °C under dark condition. After 1-day incubation, uniformly germinated seeds were selected and transferred to Petri dishes (9.0 cm) containing two sheets of Whatman No. 1 filter paper moistened with 10 mL of distilled water or test solutions. Each Petri dish contained 20 germinated seeds. Each treatment was replicated 4 times. The germinated seeds were allowed to grow at 27 °C in darkness and

3 mL of distilled water or test solutions was added to each Petri dish on day 3 of the growth. Fresh weight of roots was measured at the times indicated.

Proline was extracted and its concentration determined by the method of Bates et al. [4]. Roots were homogenized with 3% sulfosalicylic acid and the homogenate was centrifuged. The supernatant was treated with acetic acid and acid ninhydrin, boiled for 1 h and then absorbance at 520 nm was determined. Levels of proline are express as μmol per g fresh weight.

POX was extracted according to the method described by MacAdam et al. [14]. Briefly, roots were frozen in liquid N and ground with a mortar and pestle and mixed with either 0.05 M potassium phosphate buffer (pH 5.8) to extract soluble POX, or with the same buffer containing 0.8 M KCl to extract both soluble and ionically bound ("total") POX. POX activity was measured using a modification of the procedure described by Curtis [6]. The assay medium contained 0.05 M potassium phosphate buffer (pH 5.8), 7.2 mM guaiacol, 11.8 mM H_2O_2 and 0.1 mL enzyme extract in a final assay volume of 3.0 mL. The reaction was initiated by the addition of H_2O_2 and the change in absorbance at 470 nm was measured. Activity was calculated using the extinction coefficient (26.6 mM^{-1} cm^{-1} at 470 nm) for tetraguaiacol. One unit of POX was defined as the amount of enzyme that caused the formation 1 μmol tetraguaiacol per min.

3. Results

Root growth was tracked by measuring fresh weight of roots. Figure 1 shows the effects of $CdCl_2$ on growth of and proline levels in roots of rice seedlings. Increasing concentrations of $CdCl_2$ from 0.1 to 0.3 mM progressively decreased root growth and increased proline level in roots. The reduction of root growth is closely associated with the accumulation of proline in roots.

To characterize further the role of proline accumulation on Cd-induced inhibition of root growth, seeds were germinated in $CdCl_2$ solution (0.3 mM) for 3 days and, after this, the seedlings were incubated in distilled water and $CdCl_2$ (0.3 mM), respectively, for another 3 days. When 3-day-old Cd-treated seedlings were remained in $CdCl_2$, only slight root growth was observed (Fig. 2). However, when seedlings were transferred to distilled water, root growth rapidly resumed and increased linearly with increasing dura-
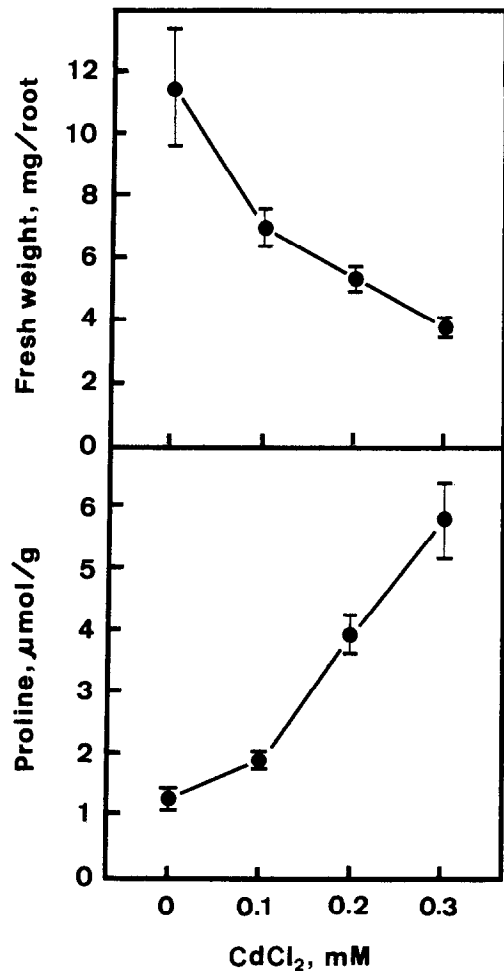

Fig. 1. Effects of $CdCl_2$ on root growth and proline level in roots of rice seedlings. Root growth and proline level were measured after 5 days of treatment. Vertical bars represent standard errors (n = 4).

tion of incubation (Fig. 2). Figure 2 also shows that proline level in roots of seedlings remained in $CdCl_2$ solution is higher than that of seedlings transferred to distilled water.

If proline accumulation is important in regulating root growth, then exogenous application of proline is expected to increase proline level in roots and to inhibit root growth of rice seedlings. As indicated in Fig. 3, this is indeed the case. Increasing concentrations of external proline from 2.5 to 10 mM progressively increased endogenous proline level and decreased root growth.

Figure 4 shows the effects of $CdCl_2$ on soluble and ionically bound POX activities in roots of rice seedlings. $CdCl_2$ had no effect on soluble POX activity
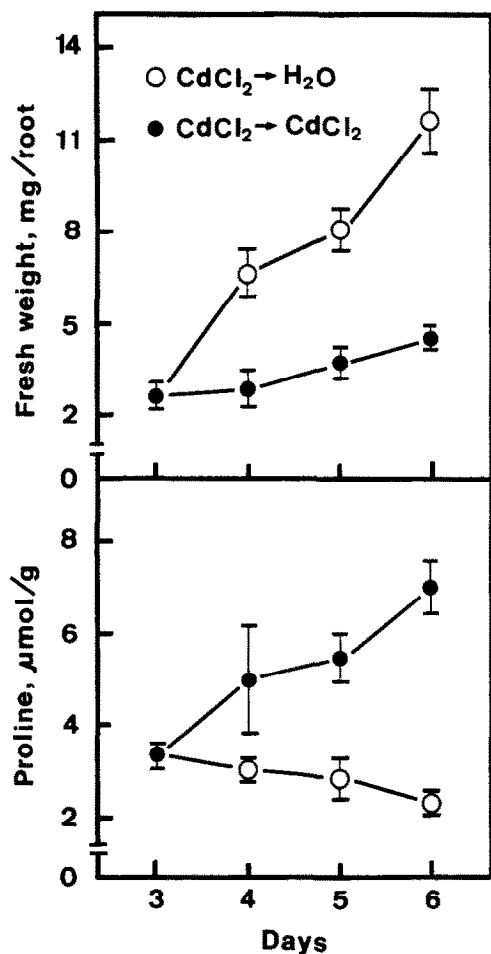
Fig. 2. Changes of fresh weight of and proline levels in roots of Cd-pretreated rice seedlings grown in the presence or absence of $CdCl_2$ (0.3 mM). Rice seeds were germinated in $CdCl_2$ (0.3 mM) for 3 days and then the seedlings were incubated in distilled water (o) and $CdCl_2$ (●), respectively. Vertical bars represent standard errors (n = 4).

Fig. 3. Effects of proline on root growth and proline level in roots of rice seedlings. Root growth and proline level was measured after 5 days of treatment. Vertical bars represent standard errors (n = 4).

Fig. 4. Effects of $CdCl_2$ on soluble and ionically bound peroxidase (POX) activities in roots of rice seedlings. Enzyme activities were measured after 5 days of treatments. Vertical bars represent standard errors (n = 4).

in roots. However, increasing concentrations of $CdCl_2$ progressively increased ionically bound POX activity. Similar to the effect of $CdCl_2$, proline treatment also resulted in an increase in POX activity in roots (Fig. 5).

4. Discussion

In rice, proline accumulation has been demonstrated in water-stressed and senescing detached leaves [12, 18] and in NaCl-stressed seedling roots [16]. The present investigation shows that proline accumulation
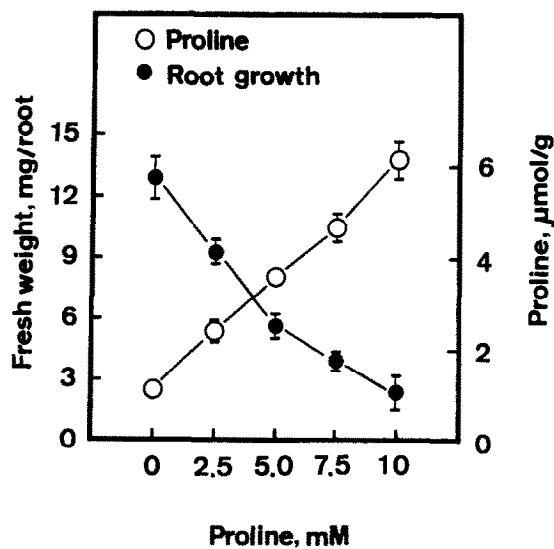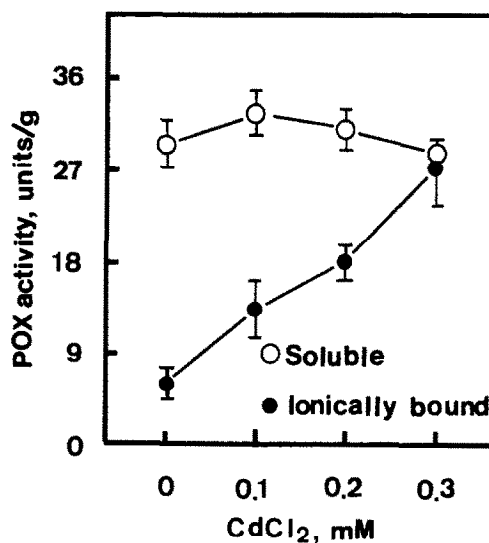
can be induced in roots by treating seedling with Cd. The ability of Cd to accumulate proline has also been observed in detached rice leaves (unpubl. data). Our results are in general consistent with those reported by Saradhi and Saradhi [17], who showed that heavy
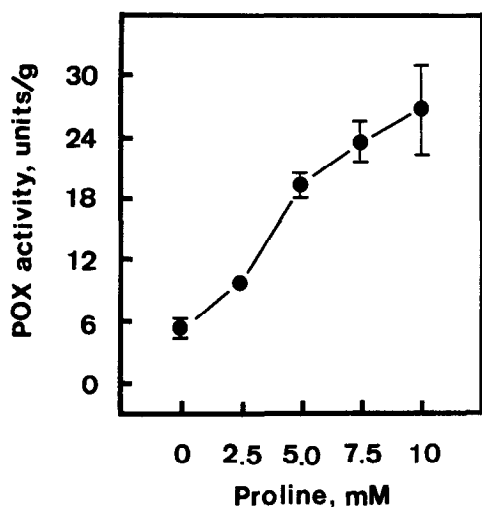
Fig. 5. Effects of proline on ionically bound peroxidase (POX) activity in roots of rice seedlings. Enzyme activities were measured after 5 days of treatments. Vertical bars represent standard errors (n = 4).

metals including Cd, can induce proline accumulation.

The present investigation also shows that proline accumulation is closely associated with growth inhibition induced by Cd. It seems that Cd-induced inhibition of root growth is a consequence of proline accumulation, since Cd-induced inhibition of root growth was produced in rice seedlings fed with proline. When rice seedlings pretreated with Cd were transferred to distilled water, root growth resumed and proline level in roots declined. These results suggest that Cd-induced inhibition of root growth is not irreversible and further supports the close link between proline accumulation and growth inhibition of roots induced by Cd. Proline accumulation induced by NaCl has also been demonstrated to be correlated with growth inhibition of *Brassica napus* callus [5].

Cd-induced increase in soluble and ionically-bound POX has previously been reported for bean leaves [9]. However, we showed that ionically bound, but not soluble, POX activity in roots was increased by Cd treatment. Ionically bound POX has been equated with the cell wall fraction because it is the activity that remains associated with cell walls of homogenized tissue until the tissue is extracted with high ionic strength buffers. It has been postulated by several investigators that the action of POX located in cell walls would be to confer rigidity to the cell wall and prevent later expansion involved in growth [7, 8, 10,

11, 14]. Thus, Cd-induced inhibition in root growth of rice seedlings is likely to result from cell wall tightening processes related to formation of cross-linkages among cell wall polymers.

Of particular interesting is the finding that proline resulted in an increase in ionically bound POX activity in roots of rice seedlings in the absence of Cd. These results, again, support our suggestion that Cd-induced proline accumulation is associated with growth inhibition of roots. However, more studies are required to confirm the control of ionically bound POX activity by proline, in particular focusing on the location of proline in cell walls.

Proline accumulation in plants under stress conditions has been suggested to play several roles [3]. Since growth is generally thought to be an energy-required process, elevated proline level induced by Cd is most likely acting as a way to save energy by inhibiting root growth, possibly through the action of POX located in cell walls, and as a readily utilizable source of energy and amino groups once Cd-stress is relieved.

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