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Glutathione reduces the inhibition of rice seedling root growth caused by cadmium

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Abstract

We investigated the effects of agents known to affect cellular glutathione (reduced form, GSH) levels on the growth of rice seedlings treated with Cd. $CdCl_2$ was more effective than $CdSO_4$ in inhibiting root growth. However, $CdCl_2$ had no effect on shoot growth. GSH, a substrate for phytochelatin synthesis, was effective in counteracting growth inhibition of roots by $CdCl_2$. Root growth in the $CdCl_2$ medium was found also to be enhanced by the addition of L-glutamic acid and L-cysteine, both of which are substrates for GSH formation. Buthionine sulfoximine, an inhibitor of GSH synthesis, rendered the roots susceptible to growth inhibition by Cd. Our results suggest that GSH level may play a role in regulating Cd-inhibited growth of rice roots.

Abbreviations: BSO = buthionine sulfoximine; GSH = reduced form glutathione

1. Introduction

Cd is one of the most toxic among the heavy metals. It is supplied to soil, air and water mainly by effluent from industries, mining, burning and leakage of waste, and by fertilization with phosphates and sewage sludge. In Taiwan, Cd poses a serious problem for rice production. Cd interferes with seedling growth [1, 8]. Plant cells subjected to Cd rapidly synthesize phytochelatins, i.e. Cd-binding peptides, whose function is to sequester and to detoxify excess Cd ions [10, 14]. Failure to synthesize these peptides results in growth inhibition and cell death [10, 14]. Phytochelatins are synthesized from GSH [4, 13]. It has been reported that BSO, an inhibitor of GSH biosynthesis [3], caused no reduction of growth of tobacco cells in the absence of Cd, but growth was greatly reduced in cultures exposed to BSO and Cd [11]. Hence it is of interest to study the effects of agents known to affect cellular GSH levels on the Cd-inhibited root growth of rice seedlings.

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 thoroughly 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 growth. Length and fresh weight of roots and shoots were measured after 5 days in darkness. All experiments described here were repeated three times. Similar results of identical trends were obtained each time. The data reported here are from a single experiment.

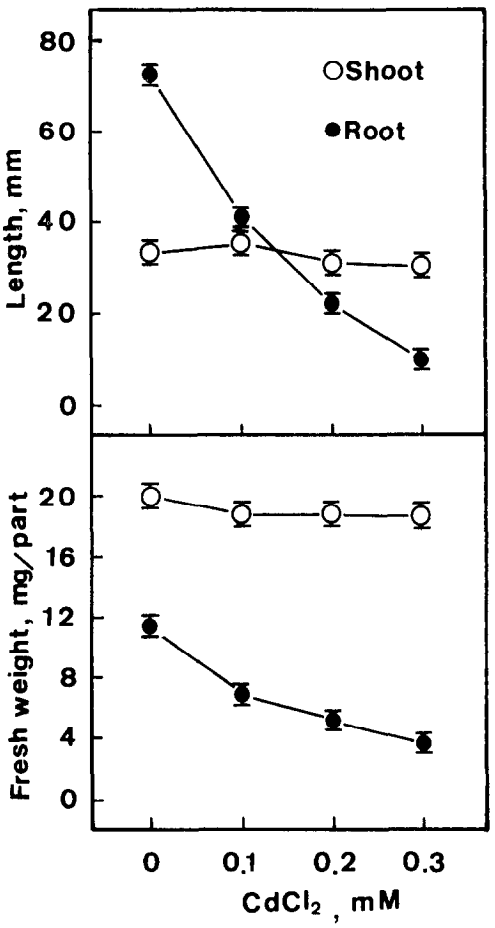

Fig. 1. Effects of $CdCl_2$ on the growth of rice seedlings. Seedling growth was measured after 5 days of treatment. Vertical bars represent standard errors.

3. Results and discussion

Figure 1 shows the effect of $CdCl_2$ on the growth of rice seedlings. $CdCl_2$ significantly reduced root growth, as judged by root length and root fresh weight, of rice seedlings. Increasing concentrations of $CdCl_2$ from 0.1 to 0.3 m*M* progressively decreased root growth. However, no reduction of shoot growth by $CdCl_2$ was observed. The differential effect of Cd on root and shoot growth could be accounted for by the fact that Cd is accumulated mainly in roots and to a minor extent in shoots [5, 6, 15]. Our results indicate that the primary effect of Cd is to take place in roots of rice seedlings.

When the effect of $CdSO_4$ on root growth of rice seedlings was compared with that of $CdCl_2$, it was found that $CdSO_4$ was less effective in reducing root growth (Fig. 2). Sulphate has been shown

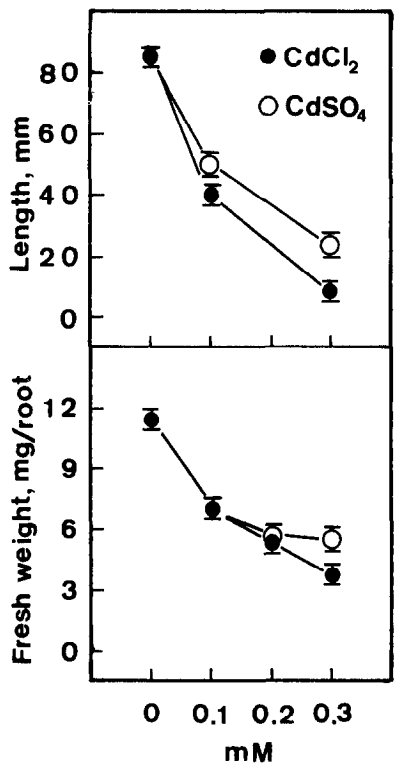

Fig. 2. Effects of $CdCl_2$ and $CdSO_4$ on root growth of rice seedlings. Root growth was measured after 5 days of treatment. Vertical bars represent standard errors.

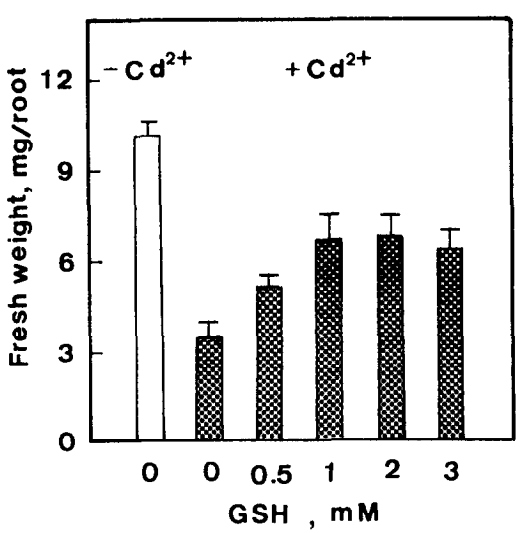

Fig. 3. Effects of GSH on root growth of rice seedlings in the presence of $CdCl_2$ (Cd^{2+}, 0.3 m*M*). Root growth was measured after 5 days of treatment. Vertical bars represent standard errors.

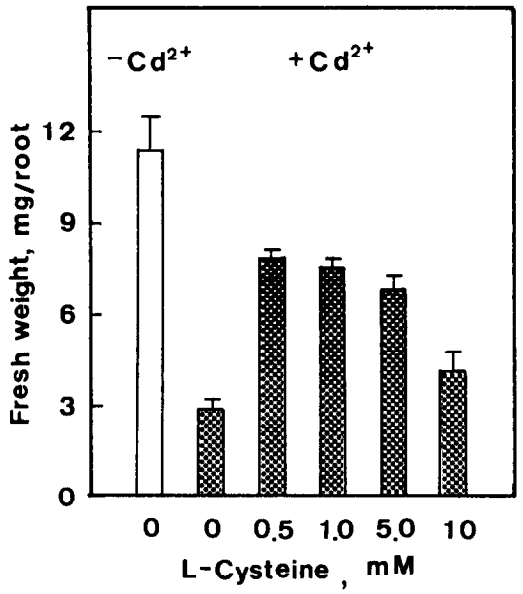

Fig. 4. Effects of L-cysteine on root growth of rice seedlings in the presence of $CdCl_2$ (Cd^{2+}, 0.3 m*M*). Root growth was measured after 5 days of treatment. Vertical bars represent standard errors.

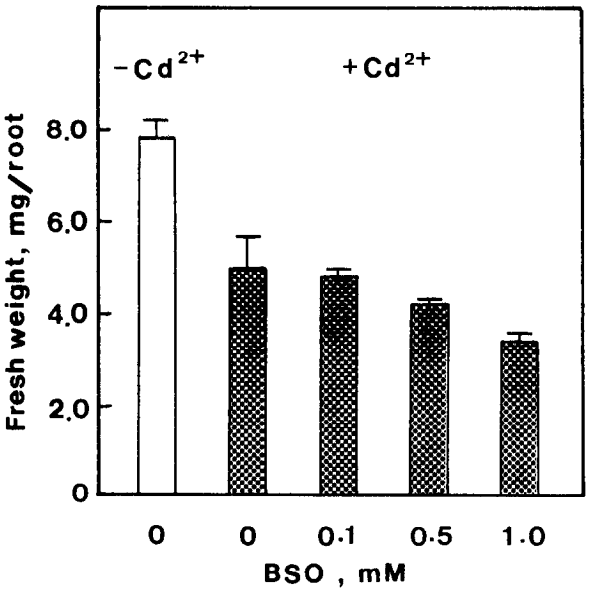

Fig. 6. Effect of BSO on root growth of rice seedling in the presence of $CdCl_2$ (Cd^{2+}, 0.1 m*M*). Root growth was measured after 5 days of treatment. Vertical bars represent standard errors.

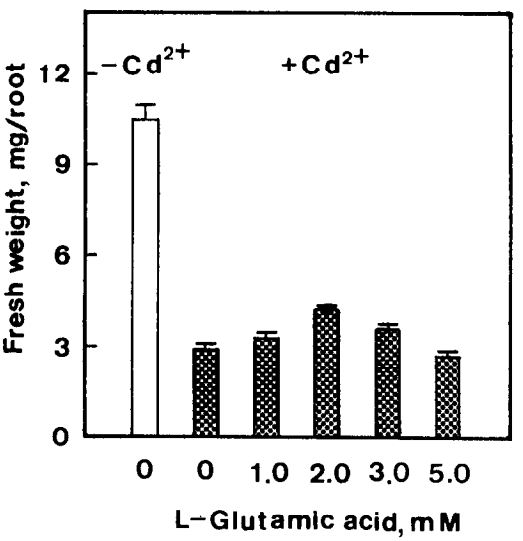

Fig. 5. Effects of L-glutamic acid on root growth of rice seedlings in the presence of $CdCl_2$ (Cd^{2+}, 0.3 m*M*). Root growth was measured after 5 days of treatment. Vertical bars represent standard errors.

to induce accumulation of GSH [2]. If high levels of GSH play an important role in regulating Cd-induced growth inhibition of rice roots, then the growth of roots in $CdCl_2$ is expected to be enhanced by adding GSH. As indicated in Fig. 3, root growth was indeed significantly improved by GSH in the $CdCl_2$ treatment. Additional experiments were conducted to see if glycine, L-glutamic acid and L-cysteine, all of which are substrates for GSH synthesis [12], can also reduce growth inhibition by $CdCl_2$. Root growth in the $CdCl_2$ medium was indeed enhanced by the addition of L-glutamic acid or L-cysteine (Figs. 4 and 5). L-Cysteine is even more effective than L-glutamic acid in counteracting growth inhibition by $CdCl_2$. However, glycine had no effect on Cd-inhibited root growth (data not shown), suggesting that the glycine level is sufficient for GSH synthesis in Cd-treated roots.

Since D-cysteine is ineffective in counteracting growth inhibition of roots by $CdCl_2$ (data not shown), the observed positive effect of L-cysteine on the recovery of root growth in the presence $CdCl_2$ is unlikely to be due to binding of Cd with the cysteine thiol, leading to a reduced level of Cd in the medium.

To further confirm the beneficial effect of GSH on root growth of rice seedlings in $CdCl_2$ medium, BSO, an inhibitor of GSH synthesis, was used to test its effect on root growth of rice seedlings in the presence of low concentration (0.1 m*M*) of $CdCl_2$. As indicated in Fig. 6, BSO rendered the roots susceptible to growth inhibition by Cd.

Taking all data into account, we conclude that GSH levels in roots may play an important role in regulating Cd-inhibited root growth of rice seedling. The effect of

GSH on Cd-inhibited growth of rice roots is unlikely to be due to its characteristic of being an antioxidant, since ascorbic acid was found to be ineffective in counteracting Cd-inhibited root growth (data not shown). It has been shown that GSH stimulated the accumulation of phytochelatins in Cd treated cells [7]. It seems most likely that the positive effect of GSH on root growth is mediated through the synthesis of phytochelatins, which leads to a reduced level of free Cd ions in root cells. Clearly a deeper knowledge of GSH-induced phytochelatin accumulation in rice roots is necessary for a better understanding of Cd-inhibited growth of rice roots.

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