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Short Communication

Reversal of Chlorsulfuron-Induced Inhibition of Mitotic Entry by Isoleucine and Valine[1]

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THOMAS L. ROST* AND THOMAS REYNOLDS
Department of Botany, University of California, Davis, California 95616 (T.L.R.); *and Department of Biology, University of North Carolina, Charlotte, North Carolina 28223* (T.R.)

ABSTRACT

Pea (*Pisum sativum* L. cv Alaska) root tips were excised and cultured aseptically in White's medium. Cultures were treated immediately or after a 24 hour equilibration time with 28 nanomolar chlorsulfuron plus isoleucine and valine (each 0.1 millimolar), isoleucine and valine, or untreated. The percentage of mitotic figures in untreated control roots sampled immediately after excision showed a transitory drop and recovery within 24 hours (an excision effect). In chlorsulfuron-treated roots, the percentage of mitotic figures did not recover. In roots treated with chlorsulfuron plus isoleucine and valine, a complete recovery did occur. If roots were treated with chlorsulfuron 24 hours after excision, the percentage of mitotic figures was reduced to near 0 by 8 hours. In roots treated with chlorsulfuron plus isoleucine and valine, no reduction in mitotic figures occurred. The complete reversal of chlorsulfuron-inhibited mitotic entry by isoleucine and valine implicates these amino acids, in some manner, with the control of cell cycles progression.

Chlorsulfuron is a new herbicide used to control weeds in cereal crops. This chemical is rather unique in that it is a highly specific growth inhibitor (3) that selectively inhibits the progression of cells through the cell cycle (5). In pea root tips, chlorsulfuron blocks cell cycle progression in both G_1 and G_2 phases without interfering directly with mitosis or DNA synthesis (5).

Ray (4) and Chaleff and Mauvais (1) have shown that chlorsulfuron inhibits the activity of acetolactate synthase, an enzyme involved in the synthesis of the branched amino acids valine (Val), leucine (Leu), and isoleucine (Ile). Addition of Val and Ile together reversed chlorsulfuron growth inhibition in cultured pea roots and whole plants.

Except for one example, there is no known or implicated role for specific amino acids in cell cycle regulation, except for a general requirement for continuous protein synthesis (2, 9). When Chinese hamster cells (CHO) are grown in a complex serum medium including several amino acids but lacking Ile they became arrested selectively in G_1 (7, 8). Isoleucine is perhaps involved in an initiation step for DNA synthesis during the G_1 phase in cultured rodent cells.

The fact that chlorsulfuron inhibits the biosynthesis of Ile and Val and that its growth inhibition response is cell cycle specific cannot be pure coincidence. In this study, we demonstrate that Ile and Val totally reverse the inhibition of mitotic progression induced by chlorsulfuron.

MATERIALS AND METHODS

Pea seeds (*Pisum sativum* L. cv Alaska) were surface sterilized in commercial bleach for 5 min, poured on a sterile gauze extended over a large beaker, and rinsed with sterile distilled H_2O. Seeds were planted in sterile vermiculite and grown in the dark for 5 d at 26°C. Seedlings were removed and the 1 cm root tip excised and transferred into 50 ml of White's medium (10). Cultures were placed on a rotary shaker (50 rpm) at 26°C in the dark. In the first experiment, treatments were initiated immediately after excision, and in the second the roots were allowed to grow for 24 h before treatment. In both instances, chlorsulfuron at a final concentration of 28 nM, chlorsulfuron plus a 1:1 mixture of Ile and Val both at 0.1 mM, Ile and Val alone, and White's medium alone were used. Chlorsulfuron and the amino acids were prepared at higher concentrations and were filter sterilized into the culture medium to achieve the final concentrations.

Roots were sampled at intervals (4–5 per sample time) and fixed in absolute ethanol:glacial acetic acid (3:1, v/v) for at least 2 h, hydrolyzed for 20 min at room temperature in 5 N HCl, and stained with Feulgen reagent. The 2-mm tips were squashed on microscope slides and scored for the percentage of mitotic figures. All experiments were repeated twice, and at least 2000 cells from four roots were scored for each data point in each experiment. Standard errors of the mean were calculated for each point.

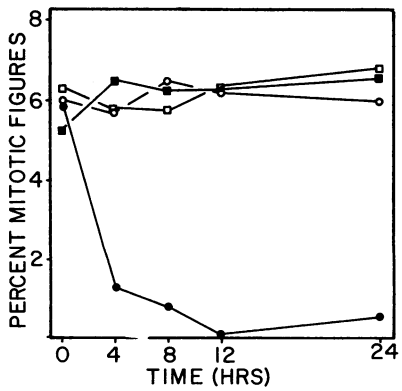

FIG. 1. Per cent mitotic figures are shown in roots excised and cultured for 24 h before treatment. Treatments are as follows: chlorsulfuron (●), chlorsulfuron plus Ile and Val (□), Ile and Val (■), and untreated control (○). Data expressed as means in which SE did not exceed 0.8.

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RESULTS

Root tips (1 cm) were excised and placed into White's medium containing chlorsulfuron, chlorsulfuron plus Ile and Val, Ile and Val, and untreated control. In the untreated control, the percentage of mitotic figures dropped rapidly so that between 4 to 8 h less than 1% of the cells were dividing. This drop was followed by a recovery to the starting value of 4 to 6%. This was a classic excision response as first reported by Wilson *et al.* (11) and it may involve wound ethylene from the cut end of the root (6). In the chlorsulfuron treatment, the percentage of mitotic figures dropped in response to the excision effect but did not recover. If the medium contained chlorsulfuron plus Ile and Val, the recovery occurred exactly as in the untreated control (data not shown). The Ile and Val treatment also responded as in the untreated control. The addition of Ile and Val completely reversed the chlorsulfuron-induced inhibition of mitotic entry.

In the second experiment, roots were excised and grown in White's medium for 24 h before treatment. This was done to insure that the excision effect period was passed and the percentage of mitotic figures restored to a constant level. The untreated control was a flat line at approximately 6% (Fig. 1), indicating that a constant number of cells were entering and leaving mitosis. In the chlorsulfuron treatment, the percentage of mitotic figures decreased rapidly and remained at near 0% for the experiment duration. This inhibitory effect was totally reversed in the chlorsulfuron plus Ile and Val treatment. Isoleucine and Val had no effect if used alone.

DISCUSSION

The mode of action of chlorsulfuron includes inhibition of growth at low concentrations without significantly inhibiting protein or RNA synthesis (3). Ray (3) also speculated that chlorsulfuron inhibited cell division by interfering with some molecular event preceding mitosis. Rost (5) examined the cell cycle response of chlorsulfuron in pea root tips and determined that the cell cycle is blocked at both G_1 and G_2. He also reported that although protein synthesis was not inhibited and RNA synthesis was only slightly reduced that chlorsulfuron could act by selectively inhibiting RNA synthesis specific to the cell cycle.

Ray (4) and Chaleff and Mauvais (1) have now reported on the specific site of action of chlorsulfuron and a related chemical, sulfometuron methyl. These herbicides act by blocking the activity of acetolactate synthase. This enzyme is necessary for one of the first steps in the biosynthesis of Val, Ile, and Leu. Ray (4) demonstrated that chlorsulfuron inhibition of pea root elongation growth can be reversed by treatment with a mixture of Ile and Val. Isoleucine alone and Leu alone or in combination with Val does not reverse growth inhibition. Valine, however, partially reversed chlorsulfuron growth inhibition.

In our study, we have demonstrated that Ile and Val completely reverse chlorsulfuron inhibition of cell entry into mitosis. Since chlorsulfuron is a cell cycle specific herbicide (3, 5), this observation provides evidence possibly implicating these amino acids as being involved in cell cycle regulation. Rost (5) showed that pea root tip cells were blocked at both G_1 and G_2. Further work on this point is required, however, to locate more specifically the primary control point affected by chlorsulfuron.

This is probably the first report to implicate an amino acid deficiency exhibiting cell cycle inhibition in plant cells. It is not the first, however, to show that Ile deficiency can affect the cell cycle. Tobey and Ley (8) and Tobey (7) observed that when CHO hamster cells were cultured in Ile-deficient medium, the cell cycle became arrested in G_1. No other phase of the cell cycle was interfered with, so that cells in S completed DNA synthesis, progressed through G_2 and divided. This progression continued through another round until all cells were arrested in G_1. After release from arrest by adding Ile, the cells resumed progression through mitosis as a synchronous wave for one cycle (7). The specific role of Ile deprivation was to inhibit the initiation of DNA synthesis. It is noteworthy that, similar to chlorsulfuron-treated pea roots, CHO cell RNA synthesis was only slightly inhibited. Protein synthesis was also not totally inhibited although some reduction in polypeptide translation rate occurred because of the lack of Ile.

Lack of Ile and Val, due to chlorsulfuron inhibition of acetolactate synthase activity, causes inhibition of root growth predominantly by stopping cell cycle progression in G_1 and G_2. This is a slightly different response than in CHO cells which arrests only in G_1. Nonetheless, the similarity in response cannot be simply coincidental and will lead to further experimentation on the possible role of branched amino acids on cell cycle regulation.

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