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"HERBICIDE RESISTANCE IN WEEDS AND CROPS"



ABSTRACTS

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22 OCCURRENCE OF CYTOCHROME P₄₅₀ MONO-OXYGENASES IN THE METABOLISM OF CHLOROTOLURON BY WHEAT MICROSOMES

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In previous studies, it has been shown that wheat cell cultures actively metabolize chlorotoluron (CPU). That metabolism was found sensitive to several cytochrome P₄₅₀ inhibitors, and could be stimulated by treatments of cell cultures with compounds such as cyometrinil, dichlormid and 2,4-D(1,2). Mono-oxygenases implicated in the metabolism of CPU were then studied in microsomal preparations from wheat cell cultures pretreated with cyometrinil.

Non-chlorophyllous cells of wheat (*Triticum aestivum* var. Koga 11) were routinely cultivated as previously described(2). At the beginning of experiments, 4-8 day-old suspensions were subcultured at an inoculum density of 4 mg dry wt/ml. After 4 days of growth, cells were put in fresh culture medium containing 400 µM cyometrinil. Microsomes were isolated 3 days later. Oxygenase activities were tested using 100,000 g pellets, referred to as microsomal fractions. Protein concentrations in these fractions were 2-4 mg/ml. Reaction mixtures, adjusted to a final volume of 200 µl with 0.1 M (pH 7.5) potassium buffer, usually contained 16.6 µBq of ¹⁴C-CPU plus cold herbicide to a final concentration of 500 µM, 1 mM NADPH, a NADPH regenerating system and 0.25 - 0.40 mg of microsomal proteins. Enzymatic reactions were stopped after 30 min with 20 µl of 4N HCl. Rates of cinnamate hydroxylase and laurate hydroxylase activities were assessed in similar conditions for comparison.

Aerobic incubations of microsomes with CPU and NADPH led to the formation of two metabolites, namely ring-methyl hydroxylated CPU, and N-monodemethylated CPU. Microsomes metabolized the herbicide at rates ranging from 600 to 650 and 200 to 250 pmoles/mg/30 min for the ring-methyl hydroxylase and N-demethylase, respectively. When cells were grown in the presence of 400 µM cyometrinil, activities were stimulated 1.6- and 3.5-fold, respectively. The cytochrome P₄₅₀ content of microsomes was increased 1.9-fold by cyometrinil, while that of cytochrome b5 was not affected.

Requirements for the *in vitro* hydroxylation and N-demethylation of CPU were determined. Both activities required O₂ and NADPH as a cofactor. Other reductants such as sodium dithionite or ascorbate were ineffective. Incubations under nitrogen atmosphere showed that molecular oxygen was necessary for the two activities. In addition, cumene hydroperoxide also supported the N-demethylase activity. At pH 7.5 and 25°C, rates of activities were almost linear up to 40 min for protein concentrations between 1.15 and 2.55 mg/ml. The CPU hydroxylase activity did not follow a Michaelian kinetic. Regarding the N-demethylase activity, data presented too much variability to allow a correct analysis. Activities were higher in the light than in the dark. Addition of CO inhibited the hydroxylase activity without any apparent effects on the N-demethylase activity. In the presence of 1-aminobenzotriazole, a suicide substrate of P₄₅₀, the hydroxylase activity was strongly reduced, as previously noted in whole plants(1) and cells(2).

These data provide the first demonstration of the in vitro oxidative metabolism of CPU by wheat cell microsomes. The hydroxylase is probably of cytochrome P₄₅₀ type, since its activity is NADPH and O₂-dependent, and is sensitive to CO in a partly reversible way by the light. These results also suggest that the N-demethylation at least partly depends on a P₄₅₀ system, since it is NADPH and O₂-dependent, and was also stimulated following treatment of cells by cyometrinil. In conclusion, it appears that two cytochrome P₄₅₀ forms present in wheat are responsible for the metabolism of a phenylurea herbicide. It will be of interest to determine if other herbicides, and xenobiotics, can also be substrates of similar enzymatic systems.

References

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