Review

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Molecular Mechanisms of Herbicide Resistance with Special Emphasis on Cytochrome P450 Monooxygenases

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1. Introduction

Herbicides can be grouped according to their sites of action and subdivided into chemical classes. Resistance to various herbicide groups has been found among over 84 different plant species (59 dicots and 25 monocots) worldwide. The extent of resistance covers the majority of known herbicide sites of action and their chemical classes. Mechanism of herbicide resistance includes; 1) modified target site, 2) enhanced detoxification, and 3) alterations in the uptake, translocation or compartmentalization of a herbicide. The first two mechanisms have mainly been identified in weeds and crops [1].

2. Modified target site

Triazine resistance is one of the most prevalent types of herbicide resistance found in weeds. In these cases, herbicide resistance is mostly due to a modification at the target site, the D1 protein of the photosystem II complex in chloroplasts, whereas crops are resistant because they can detoxify these herbicides. The D1 protein is referred to as the 32-kDa protein or the Q_B protein, and is encoded by the chloroplast *psbA* gene. Triazine and phenylurea herbicides displace plastoquinone at the Q_B binding site on the D1 protein and thereby block electron flow from Q_A to Q_B. Atrazine binding in the Q_B niche appears to be due to hydrogen bonds with Ser264 and Phe265 as well as hydrophobic interactions with Phe255. With triazine-resistant weeds, resistance is mostly due to a modification of amino acid residues in the Q_B-binding niche on the D1 protein. In all cases, resistance is a result of a point mutation of *psbA* gene, resulting in a substitution of Gly for Ser264. This modification greatly reduces the affinity of atrazine for the Q_B-binding site. Other different amino acid substitutions that confer resistance have been identified in or near the Q_B-binding niche between residues 211-275 in algae, cyanobacterium, and higher plants [1].

3. Enhanced metabolism

In most cases, crop resistance to certain herbicides is due to the ability of the crop to metabolize the herbicide and thereby prevent injury. Two enzyme systems that play major roles in conferring resistance to herbicides are glutathione S-transferase (GST) and cytochrome P450 monooxygenase consisting of cytochrome P450 (P450 or CYP) and NADPH-cytochrome P450 oxidoreductase (P450 reductase) which catalyzes oxidation reactions of lipophilic compounds including certain herbicides (**Fig. 1**).

Maize is highly resistant to triazine herbicides. Rapid detoxification of the herbicide atrazine via glutathione conjugation is the primary mechanism of resistance with three GST isozymes responsible for catalyzing the conjugation. Atrazine can also be metabolized via *N*-dealkylation. Mono-*N*-dealkylation reduces the binding affinity of atrazine for the D1 protein and thereby partially reduces its phytotoxicity. However, removal of both *N*-alkyl groups is necessary for complete detoxification. As a mechanism for herbicide resistance, *N*-dealkylation

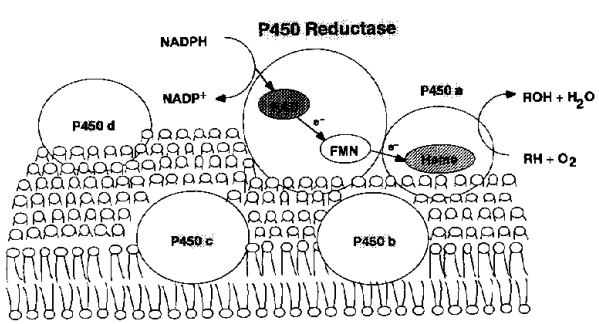

Fig. 1 Cytochrome P450 monooxygenases in plant microsomes.

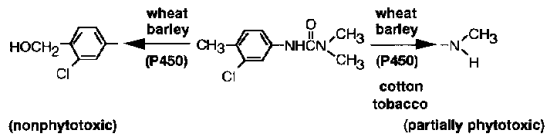

Fig. 2 Metabolism of chlortoluron in plants.

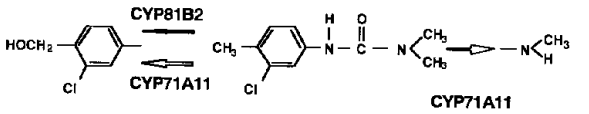

Fig. 3 Metabolism of the herbicide chlortoluron in tobacco cultured S401 cells treated with 2, 4-D.

Table 1. Some of P450-dependent oxidations of herbicides characterized in plant microsomes.

Herbicide	Oxidation
Bentazone	Aryl-hydroxylation
Chlortoluron	*N*-dealkylation, hydroxylation
Chlorsulfuron	Aryl-hydroxylation
Diclofop	Aryl-hydroxylation
Fluometuron	*N*-dealkylation, hydroxylation
Linuron	*N*-dealkylation
Metolachlor	*O*-dealkylation
Monuron	*N*-dealkylation
Primisulfuron	Aryl-hydroxylation
Triasulfuron	Aryl-hydroxylation

Table 2. Plant P450 species metabolizing herbicides.

P450	Substrate	
	endogenous	exogenous
CYP71A11	—	chlortoluron
CYP73A1	—	chlortoluron
CUP76B1	—	chlortoluron
CYP81B1	fatty acids	chlortoluron
CYP81B2	—	chlortoluron
CYP86A1	fatty acids	DCMU (?)

is considerably less efficient than is glutathione conjugation. Although P450 is thought to catalyze *N*-dealkylation, this has not been demonstrated *in vitro*. The phenylurea herbicide chlortoluron has been used for selective weed control in wheat, which can detoxify chlortoluron via ring-methyl hydroxylation and *N*-demethylation. Both reactions appear to be mediated by P450. Metabolism via ring-methyl hydroxylation is primarily responsible for chlortoluron resistance in wheat. On the other hand, *N*-demethylation makes a relatively minor contribution towards chlortoluron resistance, since cotton and tobacco metabolized the herbicide via the reaction (**Fig. 2**) [1]. **Table1** lists some of P450-dependent oxidation reactions of herbicides which have been established in plants [2, 3].

4. Plant P450 species metabolizing herbicides

P450 enzymes are involved in the metabolism of herbicides in plants. These P450 species play an important role in herbicide selectivity and resistance. However, molecular information on these P450 species was quite limited, since it was rather difficult to identify a function of a P450 species in a large gene family. It was found that tobacco cultured S401 cells treated with 2,4-D metabolized chlortoluron to give ring-methyl hydroxylated and *N*-demethylated metabolites, whereas the cells barely produced these metabolites without 2,4-D treatment. Based on these results, we attempted to clone cDNAs for P450 species metabolizing chlortoluron in tobacco cultured S401 cells treated with 2,4-D. As a result, four novel P450 cDNA clones were isolated. Based on their sequences, these were named as CYP71A11, CYP81B2, CYP81C1 and CYP81C2. These P450 species cloned were found to be in type A, which were mostly related to the biosynthesis of phenylpropanoids and other secondary metabolites [4].

Northern blot analysis of the cloned cDNAs revealed that both CYP71A11 and CYP81B2 were inducible in the S401 cells with 2,4-D treatment. Therefore, we examined the yeast *Saccharomyces cerevisiae* to see if both cDNA clones were expressed. Both CYP71A11 and CYP81B2 expressed in the yeast together with yeast P450 reductase or tobacco P450 reductase and showed higher 7-ethoxycoumarin *O*-deethylase activity than that of the control yeast strain. In addition, the yeast cells expressing both CYP71A11 and yeast P450 reductase exhibited enhanced ring-methyl hydroxylation and *N*-demethylation towards chlortoluron, whereas the yeast cells expressing both CYP81B2 and tobacco P450 reductase showed a slightly enhanced ring-methyl hydroxylation. Therefore, both CYP71A11 and CYP81B2 were found to be involved in the metabolism of chlortoluron in the S401 cells treated with 2,4-D, as shown in **Figure 3** [5]. Recently, it was reported that CYP73A1, CYP76B1, CYP81B1 from plants also metabolized chlortoluron, although fatty acids were endogenous substrates for CYP81B1 as listed in **Table 2** [7].

5. Mammalian P450 species metabolizing herbicides

Commercial herbicide chemicals have been examined for metabolism in mammals from the standpoint of safely assessment prior to marketing. Cytochrome P450 monooxygenases in mammalian liver

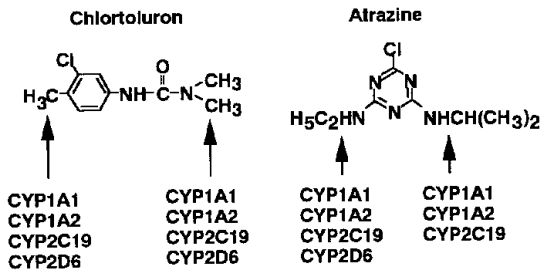

Fig. 4 Metabolism of herbicides in mammalian P450 species.

microsomes well known as drug-metabolizing enzymes involved in oxidative metabolism of xenobiotics including herbicides. However, which P450 species catalize oxidative reactions of any one herbicide has not yet been identified.

We examined expression of mammalian P450 species in the yeast and metabolism of herbicide chemicals in recombinant yeast strains. As shown in **Fig 4**, chlortoluron and atrazine were found to be metabolized through ring-methyl hydroxylation and *N*-demethylation, and *N*-dealkylation, respectively, by several mammalian P450 species. For example, human CYP1A1 catalyzed both ring-methyl hydroxylation and *N*-demethylation as well as *N*-dealkylation of atrazine [8, 9]. It was also found that the specific activity of human CYP1A1 expressed in the yeast towards chlortoluron was higher than those of tobacco CYP81B2 and CYP71A11.

6. Genetically engineered herbicide-resistant plants

Since mammalian CYP1A1 (P4501A1) metabolized chlortoluron via ring-methyl hydroxylation and *N*-demethylation, and atrazine via *N*-dealkylation at a higher rate than tobacco CYP81B2 and CYP71A11 and showed a broad substrate specificity, we attempted to express rat CYP1A1 cDNA under the control of CaMV 35S promoter and NOS terminator in potato plants and rat CYP1A1/yeast P450 reductase fused enzyme in tobacco plants (**Fig. 5**).

Both plants expressing the corresponding enzymes, which were mainly located on the microsomes, exhibited a higher P450-dependent monooxygenase

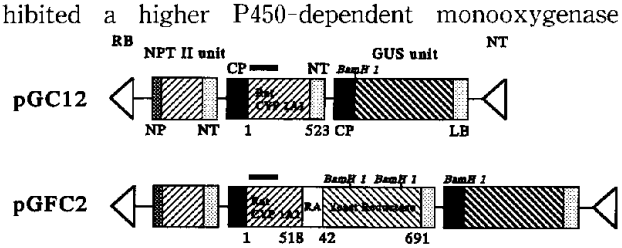

Fig. 5 The structures of the expression plasmids pGC12 for rat CYP1A1 cDNA and pGFC2 for rat CYP1A1 and yeast P450 reductase fused gene.

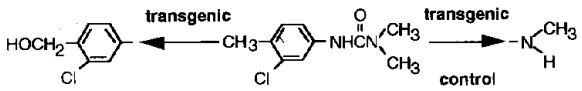

Fig. 6 Metabolism of chlortoluron in both transgenic and control tobacco plants.

activity towards 7-ethoxycoumarin than did the control plants *in vitro*. The transgenic plants expressing rat CYP1A1 and its fused enzyme showed resistance to chlortoluron. ^{14}C-Chlortoluron added to a nutrient solution was taken up by both transgenic and control plants similarly. Analysis of ^{14}C-metabolites revealed that the transgenic plants metabolized the herbicide more rapidly than did the control plants via both ring-methyl hydroxylation and *N*-demethylation (**Fig. 6**). Enhanced ring-methyl hydroxylation in the transgenic plants appeared to be responsible for resistance to the herbicide. These transgenic plants also exhibited resistance to the herbicides atrazine and pyriminobac-methyl. Thus, it was found that the expression of single CYP1A1 in plants conferred cross-resistance to the herbicides with different structures and modes of herbicide action. [10-14].

7. Concluding remarks

Cytochrome P450 monooxygenases play an important role in herbicide selectivity and resistance in plants. The plant P450 species metabolizing the herbicide chlortoluron in tobacco cultured S401 cells treated with 2,4-D were identified as CYP71A11 and CYP81B2. On the other hand, several mammalian P450 species including human CYP1A1 were found to metabolize chlortoluron as well as atrazine. So, it was suggested that in plants and mammals, different species of P450 enzymes catalyzed the same metabolic reactions towards the herbicide chemicals, although the specific activity of human CYP1A1 towards chlortoluron was higher than that of the plant enzymes.

The yeast and plant expression systems for P450 species metabolizing xenobiotics from plants and mammals were useful for comparative metabolism of the chemicals between plants and mammals. Particularly, the systems expressing human P450 species are important models for human metabolism of pesticides.

The expression of human CYP1A1 in potato plants conferred cross-resistance to the herbicides chlortoluron and atrazine. The transgenic plants expressing P450 species metabolizing xenobiotics appears to be useful for crop breeding with herbicide resistance and low pesticide residues. These plants are also important for phytoremediation.

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