METABOLISM OF HERBICIDES BY CYTOCHROME P450 IN CORN

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SUMMARY

Cytochrome P450 monooxygenases (P450) have long been considered a potentially important enzyme system for the detoxification of herbicides in corn and other crop plants. Only recently has herbicide metabolism by P450 in corn microsomes been conclusively demonstrated. Fourteen herbicides in six chemical families have been shown to be metabolized by P450 in corn. P450 is now considered of equal importance to glutathione S-transferases for the metabolism of herbicides in corn. P450 metabolism of herbicides in corn is characterized as an inducible enzyme activity which primarily catalyses hydroxylations and demethylations of the herbicide substrates. Induction of the herbicide metabolizing activity is specific, with only slight changes in the total P450 level accompanying several-fold induction of herbicide metabolism. Turnover rates for herbicide metabolism can equal those for drug metabolism. Microsomes isolated from both shoots and roots metabolize herbicides, although the majority of activities are most fully characterized in microsomes isolated from shoots of young etiolated seedlings. Many questions concerning the P450 metabolism of herbicides in corn remain. These include determining how many P450s are involved in herbicide metabolism and how their levels are regulated. Evidence is gathering that, for a number of the herbicides presently known to be metabolized by P450 in corn, there may be relatively few or even one P450 which metabolizes all the herbicide substrates.

KEY WORDS

herbicides, corn, cytochrome P450

INTRODUCTION

Herbicides employed to manage weed populations are the most heavily used class of pesticides world-wide. In 1993 they accounted for 56% and 46% of the pesticides used, representing expenditures of 4.756 and 11.70 billion dollars, in the U.S. and the world, respectively /1/. Corn is the single most important crop on which herbicides are used in the United States. Five of the ten most heavily used pesticides in U.S. agricultural production are herbicides for use in corn /1/.

Herbicides have been adopted by farmers for a number of reasons including economics, improved weed management, and flexibility in farming decisions. However, it is herbicide selectivity, where the weeds are killed and the crop is uninjured, which has made herbicides such a useful tool in modern crop production. Herbicide selectivity can be achieved by a number of methods including lower exposure, reduced absorption, more efficient metabolic detoxification, a less sensitive target site for the herbicide or a higher tolerance for the toxic effects of the herbicide in the crop compared to the weed species /2/. One or more of these mechanisms may be operative in any one case of herbicide selectivity. However, the majority of herbicide selectivity is based upon the rapid metabolism of the parent herbicide to non-toxic derivatives in the crop plant but not the weed.

Plants metabolize herbicides and other foreign organic molecules by processes which are similar to those for drugs in animal species /3/. Herbicides first undergo Phase I reactions (oxidation, reduction, hydrolysis) followed by Phase II conjugations. A fundamental difference between plants and animals is that whereas Phase II metabolites are excreted in animals, Phase II metabolites undergo further metabolism in plants (Phase III) and are often incorporated into insoluble plant components. Phase I reactions result in the complete detoxification of the herbicide in most cases but for some herbicides a Phase II reaction is needed to fully detoxify the herbicide. In these cases, the Phase I metabolite is still toxic but generally much less than the parent herbicide /4,5/.

Historically, conjugation of the parent herbicide or a Phase I metabolite with glutathione via the action of a glutathione S-transferase (GST) enzyme was considered the most important pathway for detoxification of herbicides in corn. The three most heavily used herbicide groups in corn, triazines (e.g. atrazine), acetanilides (e.g.

metolachlor) and carbamothioates (e.g. EPTC) are all detoxified through glutathione conjugation. Chemical names for the herbicides discussed are given in Table 1.

The contribution of P450 to herbicide selectivity in corn was only recently conclusively established. Hatzios and Penner /6/ in a 1982 review listed 26 herbicides, six for use in corn, suspected of being metabolized by P450. The involvement of P450 was suggested by the types of reactions that the herbicides underwent. However, at that time (1982), only three herbicides had been shown to be oxidized by P450 in a microsomal system. The demethylation of methlyurea herbicides in microsomes isolated from cotton was the first demonstration of the involvement of a plant P450 in herbicide detoxification /7/.

In contrast, since the demonstration in 1990 of P450 metabolism of a herbicide (bentazon /8/) in microsomes isolated from corn, every major new group of herbicides introduced for weed management in corn is known to be detoxified, wholly or partially, through the action of P450 (see below). In addition, several older herbicides have also been shown to be metabolized by P450 in corn microsomes.

A number of reasons may have contributed to this change in the appreciation of the role of P450 in herbicide selectivity in corn. First, the nature of the herbicide chemistry involved has changed. In a trivial sense, the more recently introduced herbicides may simply be better substrates for corn P450 isozymes than older ones. A major difference between older and newer groups of herbicides is the amounts of the chemicals that are applied in the field. Whereas the older groups (triazines, acetanilides, carbamothioates) were used at rates of thousands of grams per hectare, the newer herbicides (imidazolinones, sulfonamides, sulfonylureas) are employed at grams per hectare. Lower rates may be required for P450 activity to be sufficient and rapid enough for herbicide detoxification before crop injury occurs. Secondly, researchers learned to use a combination of experimental techniques, including induction of P450 activity, improved isolation and assay procedures, plus corn lines with high endogenous or inducible levels of the P450 of interest, to demonstrate the involvement of P450 in herbicide metabolism.

TABLE 1

Common names and chemical names of herbicides discussed in the text

Herbicide common name	Herbicide Chemical Name
AC 263,222	(±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-methyl-3-pyridinecarboxylic acid
Atrazine	6-chloro-*N*-ethyl-*N*′-(1-methylethyl)-1,3,5-triazine-2,4-diamine
Bentazon	3-(1-methylethyl)-(1*H*)-2,1,3-benzothiadiazin-4(3*H*)-one 2,2-dioxide
CGA-152005	1-(4-methoxy-6-methyl-triazin-2-yl)-3-[2-(3,3,3-trifluoropropyl)-phenylsulfonyl]urea
Chlorimuron-ethyl	2-[[[[(4-chloro-6-methoxy-2-pyrimidinyl)amino]carbonyl]aminolsulfonyl]benzoic acid
Chlorsulfuron	1-(2-chlorophenylsulfonyl)-3-(4-methoxy-6-methyl-1,3,5-triazin-2-yl)urea
Chlortoluron	3-(3-chloro-4-methylphenyl)-1,1-dimethylurea
EPTC	S-ethyl dipropylcarbamothioate
Flumetsulam	N-[2,6-difluorophenyl]-5-methyl(1,2,4)-triazolo[1,5*a*]-pyrimidine-2-sulfonamide
Imazethapyr	(±)-2-[4,5-dihydro-4-methyl-4-(1-methylethyl)-5-oxo-1*H*-imidazol-2-yl]-5-ethyl-3-pyridinecarboxylic acid
Linuron	3-(3,4-dichlorophenyl)-1-methoxy-1-methylurea
Metolachlor	2-chloro-*N*-(2-ethyl-6-methylphenyl)-*N*-(2-methoxy-1-methylethyl)acetamide
Nicosulfuron	2-[[(4,6-dimethoxypyrimidin-2-yl)aminocarbonyl]aminosulfonyl]-*N*,*N*-dimethyl-3-pyridinecarboxamide
Primisulfuron	2-[[[[4,6-bis(difluoromethoxy)-2-pyrimidinyl]amino]carbonyl]sulfonyl]benzoic acid
Triasulfuron	1-(2-chloroethoxyphenylsulfonyl)-3-(6-methoxy-4-methyl-1,3,5-triazin-2-yl)urea

HERBICIDES METABOLIZED BY P450 IN CORN

Since the demonstration of P450 metabolism of bentazon by corn microsomes in 1990 /8/, an additional 13 herbicides, representing a total of six classes of herbicide chemistry, have been shown to be metabolized by this enzyme (Table 2). Some of these herbicides (bentazon, acetochlor, metolachlor, flumetsulam, CGA-152005, nicosulfuron, and primisulfuron) are detoxified efficiently enough by corn *in vivo* to enable their use for weed management in corn production. However, for acetochlor and metolachlor, detoxification by GST is more important for the tolerance of corn to these herbicides than the activity of cytochrome P450 /3,17,18/. The remainder of the herbicides in Table 2, despite their metabolism by cytochrome P450, will injure corn. The activity of the P450 may not be rapid enough *in vivo* to avoid herbicide damage. In some cases (chlorsulfuron and imazethapyr), it is established that the primary limitation is not lack of P450 activity but conjugation of the oxidized product to achieve complete detoxification /4,5/.

Reactions catalyzed by P450 include a number of ring hydroxylations, alkyl hydroxylations and N and O demethylations of the herbicides. These are all typical P450 reactions. In addition to the herbicides discussed, organophospate insecticides (diazinon /11/ and malathion /19/) are desulfurated by P450 in corn microsomes. The desulfurated metabolites are actually the active insect toxicant. This is the same reaction that can occur in insects and mammals /20/.

The herbicide EPTC and MG-191, a herbicide safener (a chemical which prevents the toxic effect of a herbicide on a crop), are also metabolized in corn microsomes but these appear not to be P450 mediated reactions /12/. The oxidation of EPTC to its sulfoxide was shown to be catalyzed by a peroxygenase /21/.

INDUCTION OF P450 ACTIVITY IN CORN

All of the P450 activities described above were characterized using microsomes isolated from induced corn tissue. Constitutive P450 activity can be demonstrated for many of these herbicides but the level of activity is generally too low to permit detailed experimentation. It is not clear whether the induced and constitutive activity represent action of the same P450(s). This analysis is hampered by the lack of data on

TABLE 2

Herbicides demonstrated to be metabolized by cytochrome P450 monooxygenase in corn microsomes

Herbicide common name	Herbicide family	Products formed	Reference
Bentazon	unclassified heterocycle	hydroxyphenyl	8,9
Chlortoluron	phenylurea	N-demethyl hydroxyphenyl	10
Linuron	phenylurea	N-demethyl N-methoxy	11
Acetochlor	acetanilide	unknown	12
Metolachlor	acetanilide	O-desmethyl	11
Flumetsulam	sulfonamide	hydroxyphenyl hydroxymethyltriazolo	13
CGA-152005	sulfonylurea	hydroxyphenyl	11
Chlorimuron-ethyl	sulfonylurea	hydroxypyrimidine	14
Chlorsulfuron	sulfonylurea	hydroxyphenyl	11
Nicosulfuron	sulfonylurea	hydroxypyrimidine	11,15
Primisulfuron	sulfonylurea	hydroxypyrimidine hydroxybenzoic acid	11,16
Triasulfuron	sulfonylurea	hydroxyphenyl	11
AC 263,222	imidazolinone	hydroxymethylpyridine	unpublished
Imazethapyr	imidazolinone	hydroxyethylpyridine	unpublished

the uninduced activity. Kinetic analysis of constitutive and induced P450 bentazon hydroxylase activity in sorghum species suggested that the induced and constitutive activities could represent separate enzymes /22/. Also, we have generated evidence that there are both inducible and non-inducible forms of P450 bentazon hydroxylase in corn (unpublished data). Induction of P450 in mammalian tissues by

various chemical treatments has been shown since the earliest time of working with this enzyme.

The most commonly used treatment for induction is treatment of the corn seed, prior to germination, with the herbicide safeners naphthalic anhydride (1,8-naphthalic anhydride, NA) or CGA 154281 (4-[dichloroacetyl]-3,4-dihydro-3-methyl-2H-1,4-benzoxazine, Table 3). NA was one of the first herbicide safeners developed and it can

TABLE 3

Examples of induction of cytochrome P450 monooxygenase activities and levels in microsomes isolated from corn shoots

Substrate	Inducer[1]	Activity induction	P450 induction	Reference
Chlorimuron-ethyl	NA	25-40x	Not reported	14
Primisulfuron	CGA 154281	15x[2]	2.3x	16
Flumetsulam	NA	2.0x	1x	13
	Ethanol	2.1x	1x	13
	NA + Ethanol	5.3x	1x	13
Acetochlor	Acetochlor	1.1-1.3x[3]	0.7-1.3x	12
	NA	1.8-2.4x	0.7-1.0x	12
	MG-191	2.4-3.2x	1.1-1.6x	12
	PBO	1.3-1.4x	0.8-1.1x	12
Chlortoluron	CGA 154281	15x	2x	10
Cinnamic acid	NA	1.0x	1.1x	11
Lauric acid	NA	1.7x	1.1x	11

[1]Naphthalic anhydride (NA) treatment of seed, 0.25-0.5% w/w. CGA 154281 treatment of seed, 0.02-0.05% w/w. Ethanol applied to corn seedlings as a 10% (v/v) solution 24 h prior to microsome isolation. Microsomes isolated from corn seedlings watered with aqueous solutions of acetochlor (50 μM), MG-191 (100 μM) or piperonyl butoxide (PBO, 100 μM).
[2]Combined induction of both metabolic activities detected.
[3]Range of two seperate corn lines investigated.

reduce crop injury from a number of herbicides /23/. It is no longer used commercially. CGA 154281 is a recently developed safener that is used commercially to reduce the potential for metolachlor injury tc corn. Both of these safeners induce glutathione S-transferase activities as well as P450 activities /24,25/. Other treatments which can induce P450 activities for herbicide metabolism in corn include ethanol, other safeners, piperonyl butoxide (an inhibitor of P450 activity), and the herbicide substrate. Treatment with NA or CGA 154281 has given the highest levels of induction from one compound alone. Ethanol, given as an aqueous solution 24 h prior to harvest for microsome isolation, can be synergistic with NA.

The level of induction ranges from no effect with cinnamic acid hydroxylation to 40-fold with chlorimuron-ethyl hydroxylation induced by NA (Table 3). The induction appears relatively specific because, at most, the total P450 content of the tissue is doubled while increases in P450 metabolism of the herbicides are much higher.

KINETICS AND REACTION RATES OF HERBICIDE METABOLISM

The K_m and V_{max} values for several of the P450-mediated hydroxylations of herbicides in corn microsomes have been determined (Table 4). K_m values range from 50 μM to greater than 1000 μM for imazethapyr. An accurate K_m for imazethapyr could not be determined due to water solubility constraints. V_{max} values ranged from 4 to greater than 300 pmol min^{-1} mg $protein^{-1}$. Both K_m and V_{max} values are given for induced microsomal activities. It is possible that uninduced activity, especially V_{max}, could be lower. In contrast to the V_{max} values for herbicide metabolism, we have measured reaction rates in excess of 3000 pmol min^{-1} mg $protein^{-1}$ for the hydroxylation of cinnamic acid in corn microsomes (unpublished data). Others have reported rates of cinnamic acid hydroxylation of 1400 pmol min^{-1} mg $protein^{-1}$ /11/. These differences between the rates of herbicide and cinnamic acid metabolism may reflect the difference between a natural substrate for a P450 (cinnamic acid) and that of a foreign compound which is coincidentally metabolized by a P450 which has another natural substrate. Also, the level of cinnamic hydroxylase in the tissue may be higher than the herbicide-metabolizing P450s.

One way to compare P450 activity rates is by turnover numbers. Many P450 reactions are slow, with turnover rates of 1 pmol min^{-1}

pmol $P450^{-1}$ /26/. Assuming an average of 100 pmol P450 mg microsomal $protein^{-1}$ (within the range we have measured and others have reported /11,16/), gives maximum turnover rates of 0.04 to 3.7 pmol min^{-1} pmol $P450^{-1}$ (Table 4). These turnover rates are based on bulk, not specific, P450 levels. Rates as slow as 1 pmol min^{-1} pmol $P450^{-1}$ are considered rapid enough to explain drug metabolism /26/. At least in some cases with induced systems, herbicide metabolism approaches this value, although the extreme comparison is to allene oxide synthase which has a catalysis rate of approximately 80,000 pmol min^{-1} pmol $P450^{-1}$ /26/.

One of the difficulties in interpreting the relevance of *in vitro* metabolism rates to *in vivo* rates is the lack of information on the amount of P450 in the intact plant and the amount recovered during extraction. The normal pigments of a green plant make spectroscopic determination of P450 impossible. The studies cited have all used etiolated (plants grown in the dark lacking pigmentation) seedlings. P450 in plants has also been viewed as being most highly expressed

TABLE 4

Kinetic parameters of herbicide metabolism in microsomes isolated from corn shoots

Substrate	Activity[1]	Km (μM)	Vmax (pmol/min/mg)	Reference
Primisulfuron	hydroxypyrimidine	137	7	16
	hydroxyphenyl	47	4	16
Flumetsulam	hydroxyphenyl	506	NR[1]	13
	hydroxymethyl	181	NR	13
Nicosulfuron	hydroxypyrimidine	52	373	15
Chlorimuron-ethyl	hydroxypyrimidine	70	53	14
Imazethapyr	hydroxyethyl	>1000	300	unpublished

[1]Not reported

during early seedling growth as part of a defense mechanism against pathogens /27/. However, many of the herbicides discussed are applied to much older corn plants. There must be a P450(s) present when the herbicides are applied of sufficiently high activity in order for the corn to escape herbicide injury.

Only when antibody and nucleic acid probes become available will it be possible to determine *in vivo* P450 levels and the efficiency of their extraction during microsomal preparation.

TISSUE AND SUBCELLUAR LOCALIZATION

There is little known concerning the subcelluar location of the P450s which are responsible for herbicide metabolism in corn. The assumption is that they are located in the endoplasmic reticulum as are the majority of plant P450s which have been localized /28/. However, there is evidence for plant P450 activities in other subcelluar locations /28/.

The majority of the studies which have examined the metabolism of herbicides in corn have utilized microsomes isolated from shoot tissues. However, the metabolism of herbicides in the roots can be a very important factor in the ability of corn to escape herbicide injury. Many of these herbicides (linuron, acetochlor, metolachlor, flumetsulam, imazethapyr) are applied directly to the soil and others may enter the soil following an application to the plant foliage. Furthermore, these herbicides can be toxic to the root as well as to the shoot of the corn. Some of the studies examined herbicide metabolism in microsomes from both the roots and shoots.

Higher levels of primisulfuron hydroxylating activity were found in microsomes from shoots than from roots of CGA 154281 treated plants /16/. In contrast, higher levels of acetochlor metabolism were found in microsomes isolated from roots than from shoots of plants pretreated with acetochlor, naphthalic anhydride, or piperonyl butoxide /12/. Microsomes isolated from shoots or roots of plants that were not pretreated with an inducer or treated with MG-191 had similar rates of acetochlor metabolism. This suggests, at least for acetochlor metabolism by P450, that there are no constitutive differences in the level of the P450 activity between roots and shoots but, rather, in the response to inducers between the two organs, although this assumes equal recovery of P450 in the microsomes from the two plant parts.

INTERACTIONS WITH INHIBITORS AND OTHER P450 SUBSTRATES

The current status of the understanding of the numbers and relationships between the various P450 activities described for metabolizing herbicides, and in fact all plant P450s, can be compared to the early history of P450 studies in mammalian systems. Efforts are being made to use induction profiles, differential inhibitor sensitivities, and substrate interactions to delineate the various P450 activities and isozymes present in a microsomal system. These approaches can produce conflicting results and a truly clear understanding of the P450 systems in plants will require expression of isolated P450s. However, these studies have provided insight into some of the P450 isozymes in corn.

Table 5 lists the responses of various herbicide-metabolizing P450 activities as well as the hydroxylations of cinnamic acid and lauric acid to a number of P450 inhibitors. What is becoming clear is that cinnamic acid hydroxylase, and likely lauric acid hydroxylase, are separate P450 isozymes not involved in herbicide metabolism in corn. This view is based on the inhibitor profiles for these two P450 activities, particularly the low inhibition by tetcyclasis, compared to the herbicide activities, low induction with naphthalic anhydride (Table 3), and substrate interactions (unpublished and Nelson Balke, personal communication); however, a cinnamic acid hydroxylase from Jerusalem artichoke expressed at high levels in yeast can carry out a low level of herbicide metabolism (Danielle Werck, personal communication). In contrast to corn, diclofop (methyl-{RS}-2-[4-(2,4-dichlorophenoxy)-phenoxy]propanoate) and lauric acid are thought to be metabolized by the same P450 isozyme in wheat /29/.

Comparing the responses of the herbicide metabolizing activities to the inhibitors yields little information which can be used to separate them. Generally, all the activities are severely inhibited by tetcyclasis and piperonyl butoxide with less inhibition by palcobutrazol and SKF-525A. There is some variation in inhibition by 1-ABT. These data suggest that rather than a multitude of P450 isozymes which metabolize the herbicides, there may be one or a few related P450s which mediate these reactions.

An alternative to using these classic inhibitors of P450 activities is to examine the effects of other chemicals on the herbicide metabolism. Soon after the introduction of primisulfuron and nicosulfuron for weed management in corn, it was found that applications of organo-

TABLE 5

Effect of cytochrome P450 inhibitors on substrate metabolism in microsomes isolated from corn shoots

Substrate	Inhibitor[1]					Reference
	Te cy:lasis (10 µM)	1-ABT (250 µM)	PBO (100 µM)	Palcobutrazol (25 µM)	SKF-525A (250 µM)	
	% Inhibition of Metabolism					
Prim sulfuron	82[2]	39	97	---	27	11,16
Imazethapyr	78	35[3]	89[4]	32	---	unpublished
Metolachlor	98	74	75	---	28	11
Bentazon	93[5]	51	86	---	22	8,11
CGA-152005	92	73	97	---	33	11
Triasulfuron	89	63	98	---	57	11
Nicosulfuron	99	33	85	---	37	11
Flumetsulam	81-84[6]	---	56-83[7]	53-72	---	13
Cinnamic acid	0	40	0	---	11	11
Lauric acid	48	61	36	---	11	11

[1]1-ABT, 1-aminobenzotriazole; PBO, piperonyl butoxide
[2]Mean of two reported inhibitions, range 72-84% inhibition
[3]1-ABT at 1000 µM
[4]PBO at 25 µM
[5]Mean of two reported inhibitions, range 92-94%
[6]Inhibition
[7]Range of two metabolites for this one substrate
PBO at 20 µM

phosphate insecticides, particularly terbufos, resulted in injury to the corn when these normally tolerated herbicides were applied /30,31/. Inhibition of the P450 mediated detoxification of the herbicides by the insecticide was suggested by the finding that another organophosphate insecticide, malathion, inhibited the *in vitro* metabolism of primisulfuron in corn microsomes /32/. Malathion was proposed to cause the inhibition of primisulfuron metabolism through a mechanism-based or suicide substrate enzyme inactivation. We found that malathion was also an effective inhibitor of bentazon, imazethapyr and nicosulfuron metabolism in corn microsomes (data not shown). Furthermore, malathion is metabolized (desulfurated) by the corn microsomes /19/. Malathion did not inhibit cinnamic acid hydroxylation. This implies that one P450 metabolizes primisulfuron, nicosulfuron, bentazon, imazethapyr and malathion or, if there is more than one P450 for the herbicides, all of the isozymes can metabolize malathion.

Interestingly, when terbufos was tested for the ability to inhibit nicosulfuron metabolism in intact corn plants and microsomes, it was only a weak inhibitor of the metabolism /33/. It was found that the metabolites of terbufos, particularly the sulfone of terbufos, were strong inhibitors of nicosulfuron metabolism *in vivo* and *in vitro*. Further work found that terbufos sulfone inhibited the metabolism of not only nicosulfuron in corn microsomes, but also bentazon, chlorimuron-ethyl, imazethapyr and malathion, but not cinnamic acid (unpublished data). Again, this suggests that the P450s for the pesticides share common features or that there is only one P450 that metabolizes all these substrates with the exception of cinnamic acid.

We have attempted to determine whether one P450 could be metabolizing more than one substrate by testing the ability of one substrate to inhibit the metabolism of another. Nicosulfuron, chlorimuron-ethyl, chlorsulfuron, linuron, chlortoluron, bentazon, 2,4-D, dicamba, and cinnamic acid were tested for the ability to inhibit metabolism of chlorimuron-ethyl, nicosulfuron and imazethapyr (unpublished data). Chlorimuron-ethyl metabolism was strongly (›50%) inhibited by linuron and chlortoluron, moderately (~50%) inhibited by nicosulfuron, bentazon, 2,4-D and chlorsulfuron, and not inhibited by dicamba and cinnamic acid. Nicosulfuron metabolism was strongly inhibited by bentazon and chlorsulfuron, moderately inhibited by chlorimuron-ethyl, 2,4-D, and chlortoluron, and not inhibited by dicamba and cinnamic acid. Imazethapyr metabolism was strongly

inhibited by chlorimuron-ethyl, bentazon, chlorsulfuron and chlortoluron, moderately inhibited by nicosulfuron, linuron, and 2,4-D, and shghtly or not inhibited by dicamba and cinnamic acid. These data suggest that dicamba and cinnamic acid do not share a P450 in common with chlorimuron-ethyl, nicosulfuron and imazethapyr. However, the inhibitions by the other substrates imply the possibility that there is a common P450(s) among the herbicides. P450 isozymes which can metabolize multiple substrates are well established in the literature on mammalian P450s.

Additional experiments established that bentazon is a non-competitive inhibitor of nicosulfuron metabolism, nicosulfuron is a non-competitive (mixed) inhibitor of bentazon metabolism, and chlortoluron is a non-competitive inhibitor of chlorimuron-ethyl metabolism. These data imply that the substrate pairs (bentazon/nicosulfuron and chlorimuron-ethyl/chlortoluron) are sharing a common P450 but are binding at different sites.

DISCUSSION

Rapid progress has been made since 1990 in demonstrating that a number of herbicides are metabolized by P450 in microsomes isolated from etiolated corn seedlings. It is likely that the list of herbicides and other pesticides known to be metabolized by P450 will be extended. Even though the question of whether herbicides are metabolized by P450 in corn has been answered a number of questions still remain.

One intriguing question is: How many different P450s are there present in corn and how many are involved in herbicide metabolism? One school of thought is that there are a number of different isozymes responsible for herbicide metabolism. Certainly, the evidence indicates that cinnamic acid hydroxylase activity is separate from herbicide metabolism. Evidence for multiple isozymes which metabolize different herbicides is suggested by differential induction by NA, varying levels of NADH substitution for NADPH and synergism, and differences in inhibitor sensitivities of the various herbicide metabolizing activities /11/. In contrast, we might argue that a few, or even one, P450 could metabolize bentazon, nicosulfuron, chlorimuron-ethyl, imazethapyr, malathion and terbufos sulfone. It is interesting to note that the imidazolinone, sulfonamide, and sulfonylurea herbicides all inhibit the same enzyme target, acetolactate synthase. If such diverse chemistry

can all have the same target site, why not the same detoxifying enzyme(s)? A single P450 for nicosulfuron, chlorimuron-ethyl, imazethapyr, bentazon, malathion, terbufos-sulfone and, perhaps, other substrates is supported by the cross sensitivity of these activities to inhibition by terbufos sulfone. Alternate substrate inhibition of chlorimuron-ethyl, nicosulfuron, and imazethapyr metabolism also suggests some degree of competition between the substrates for a common P450. We have studied the inheritance of bentazon, imazethapyr, and nicosulfuron metabolism in corn (unpublished data). A single gene controls nicosulfuron metabolism and expression of this same gene controls bentazon and imazethapyr metabolism. The simplest explanation for this observation is that this gene controls the activity of a single P450 which can metabolize all three substrates. We have also shown that there is a second gene controlling bentazon metabolism which is associated with a P450 activity which is not induced by NA. Thus, there may be multiple substrates for one P450 and multiple P450s for one herbicide.

Other questions concerning P450 metabolism of herbicides in corn include the areas of regulation, including induction, plus the patterns of temporal and developmental expression of a particular P450(s). What is exciting about the further study of herbicide metabolism by P450 in corn is that it will not only provide information about how a plant avoids herbicide injury, but also about many aspects of basic plant biology. Perhaps the largest question is how this new knowledge will be used and applied.

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