Herbicide-Resistant Tobacco Plants Expressing the Fused Enzyme between Rat Cytochrome P4501A1 (CYP1A1) and Yeast NADPH-Cytochrome P450 Oxidoreductase

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Transgenic tobacco (*Nicotiana tabacum* cv Xanthi) plants expressing a genetically engineered fused enzyme between rat cytochrome P4501A1 (CYP1A1) and yeast NADPH-cytochrome P450 oxidoreductase were produced. The expression plasmid pGFC2 for the fused enzyme was constructed by insertion of the corresponding cDNA into the expression vector pNG01 under the control of the cauliflower mosaic virus 35S promoter and nopaline synthase gene terminator. The fused enzyme cDNA was integrated into tobacco genomes by *Agrobacterium* infection techniques. In transgenic tobacco plants, the fused enzyme protein was localized primarily in the microsomal fraction. The microsomal monooxygenase activities were approximately 10 times higher toward both 7-ethoxycoumarin and benzo[a]pyrene than in the control plant. The transgenic plants also showed resistance to the herbicide chlortoluron.

Microsomal Cyt P450 monooxygenases are composed of P450 and NADPH-Cyt P450 oxidoreductase (EC 1.6.2.4), and catalyze oxidative metabolism of a wide variety of endogenous and exogenous compounds. The catalytic versatility of P450 monooxygenase in mammals is derived from the multiplicity of P450 enzymes as well as the overlapping substrate specificity of each P450 species. Over 221 P450 genes have been identified in animals, plants, and microorganisms and have been classified into 36 gene families on the basis of sequential similarity (Nelson et al., 1993).

We have established the yeast expression systems for microsomal P450 species (Oeda et al., 1985; Yabusaki et al., 1991) and constructed chimeric P450 enzymes among different P450 species (Sakaki et al., 1987) and fused enzyme variants between rat CYP1A1 and rat reductase (Murakami et al., 1987; Yabusaki et al., 1988), bovine CYP17A and yeast reductase (Shibata et al., 1990), and bovine CYP21B and yeast reductase (Sakaki et al., 1990). The fused enzymes were catalytically self-sufficient and exhibited high specific monooxygenase activities.

P450 monooxygenase in higher plants is involved in not only the biosynthesis of a variety of secondary metabolites but also the metabolism of xenobiotics including herbicides (Durst, 1991). Oxidative metabolism mediated by P450 is particularly important for detoxification and selective toxicity

of herbicides in a variety of crop species. However, molecular studies on plant P450 species involved in the metabolism of herbicides have not been reported yet. Saito et al. (1991) reported that the transgenic tobacco plants expressing rabbit CYP2C14 showed phenotypical changes and rapid senescence but no monooxygenase activities derived from CYP2C14.

In this study we describe expression of a genetically engineered fused enzyme between rat CYP1A1 (EC 1.14.99.8) and yeast reductase in tobacco plants. Transgenic tobacco plants were analyzed for integration of cDNA into genomes, production of mRNA and protein, and monooxygenase activities. In addition, these transgenic plants were assayed for resistance to the herbicide chlortoluron.

MATERIALS AND METHODS

Chemicals and Biochemicals

DNA modifying enzymes and the Random Primer DNA Labeling Kit were purchased from Takara Shuzo Co., Ltd. (Kyoto, Japan). 7-Ethoxycoumarin, 7-hydroxycoumarin, and antibiotics were purchased from Sigma. Benzo[a]pyrene and PMSF were obtained from Wako Pure Chemical (Osaka, Japan) and Gibco/BRL (Grand Island, NY), respectively. 3-(3′-Chloro-4′- methylphenyl)-1,1-dimethylurea(chlortoluron) was given by Ciba-Geigy (Japan). 3-Hydroxybenzo[a]pyrene was a gift from Prof. T. Omura of Kyushu University (Japan). Polyclonal antibodies against yeast reductase were kindly provided by Dr. Yuzo Yoshida of Mukogawa Women's University (Japan).

Cell Culture and Plasmids

Escherichia coli HB101 [F⁻, hsd20, recA13, ara-14, proA2, LacY1, galK2, rpsL20(str), xyl-5, mtl-1, supE44, leuB6, thi-1] (Takara Shuzo, Japan), *Saccharomyces cerevisiae* AH22 (a, leu2-3, leu2-112, his4-519, can1)[cir⁺] were used as host strains. *E. coli* HB101 and *S. cerevisiae* AH22 cells were cultivated as described previously (Sakaki et al., 1990). *Agrobacterium tumefaciens* strain LBA4404 (Hoekema et al., 1983) and the plasmids pRK2013, pBI121 (Jefferson et al., 1987),

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and pBI221 were purchased from Clontech (Palo Alto, CA). *A. tumefaciens* cells were cultivated in Luria broth supplemented with 100 mg L^{-1} of rifampicin and 300 mg L^{-1} of streptomycin. *Nicotiana tabacum* cv Xanthi was grown as a shoot culture on Murashige and Skoog medium in plant boxes under fluorescent light (16 h of light) at 25°C.

The yeast expression plasmid pAFCR1 for the fused enzyme between rat CYP1A1 and yeast reductase (Sakaki et al., 1994) and the plasmid pTF2 for rat CYP1A1 were described previously (Oeda et al., 1985).

Recombinant DNA Procedures

Recombinant DNA procedures and transformation of *E. coli* were carried out as previously reported (Murakami et al., 1990). The oligonucleotide primer 1 (5'-ATGCCTTCTGTGT-ATGGATT-3'), primer 2 (5'-ACGTCTGCCAAAGCATAT-GG-3'), primer 3 (5'-AACCTACCTGTTCACTACGA-3'), and primer 4 (5'-TTACCAGACATCTTCTTGGT-3') for PCR amplification were synthesized in an Applied Biosystems (Foster City, CA) model 380A DNA synthesizer.

Plant Transformations

Recombinant plasmids were each mobilized from *E. coli* into *A. tumefaciens* using triparental mating procedures with pRK2013 as a helper plasmid (Ditta et al., 1980). Transformation of leaf discs prepared from tobacco plants with *Agrobacterium* harboring each plasmid was performed as previously described (Rogers et al., 1986). Plants resistant to 200 mg L^{-1} of kanamycin were selected.

PCR

Total DNA was extracted as described (Rogers et al., 1985) from green leaves of tobacco plants using cetyltrimethylammonium bromide as a detergent. PCR was performed with 0.5 mg of tobacco genomic DNA according to the manufacturer's instructions (Perkin-Elmer Cetus, Norwalk, CT). One set of primers 1 and 2 was designed for amplification of a 630-bp DNA fragment corresponding to the amino-terminal region of rat CYP1A1, and another set of primers 3 and 4 was for a 585-bp fragment corresponding to the carboxy-terminal region of yeast reductase. The temperature was cycled to 95°C for 1 min, then to 55°C for 1 min, and then to 75°C for 3 min for a total of 30 cycles.

For Southern blot analysis (Maniatis et al., 1989), PCR products fractionated on 1.5% agarose gels were transferred to a nylon membrane (Amersham Japan, Tokyo) and hybridized with a ^{32}P-random-labeled probe, which corresponded to either the *Hind*III fragment (3.5 kb) encoding the fused enzyme from pAFCR1 or the *Hind*III fragment (1.7 kb) encoding CYP1A1 from pTF2.

Preparation of Subcellular Fractions

Whole tobacco plants containing roots (10–15 cm height) were homogenized with a Waring blender in 2 volumes of 0.1 M potassium phosphate buffer (pH 7.4) containing 0.5 M mannitol, 5 mM EDTA, 50 mM ascorbate, 42 mM 2-mercaptoethanol, 0.1% (w/v) BSA by addition of 1 mM PMSF and

1% (w/v) PVP. Homogenates were filtered through two layers of cheesecloth and centrifuged for 15 min at 10,000g to remove cell debris. The resulting supernatants were ultracentrifuged for 60 min at 100,000g to separate a microsomal fraction from a cytosol fraction. Pelleted microsomal fractions were resuspended and homogenized in 0.1 M potassium phosphate buffer (pH 7.4) containing 30% (w/v) glycerol and 4.2 mM 2-mercaptoethanol, and stored at −80°C. Protein concentrations were determined according to the Lowry procedure (Lowry et al., 1951) using BSA as the standard.

SDS-PAGE was performed as described (Laemmli, 1970) on a 7.5 or 10% running gel. Proteins in subcellular fractions were subjected to electrophoresis and electroblotted (Towbin et al., 1979) onto PVDF-Plus (Micron Separations, Westboro, MA). Protein bands were detected using primary polyclonal antibodies against rat CYP1A1 or yeast reductase and secondary antibody conjugated with alkaline phosphatase (Pierce, Rockford, IL).

Enzyme Assays

7-Ethoxycoumarin *O*-deethylation and benzo[*a*]pyrene 3-hydroxylation activities of microsomal fractions (3 mg of protein) prepared from transgenic tobacco plants were measured as described previously (Oeda et al., 1985; Sakaki et al., 1985). For enzyme inhibition, microsomal fractions were preincubated on ice for 15 min with either anti-reductase Ig or preimmune Ig fractions (0.25 mg). Reaction mixtures containing microsomal fractions were also saturated with CO for 1 min and then assayed for enzyme activity. GUS histochemical staining and fluorometric GUS activity were performed essentially as previously described (Jefferson, 1987; Jefferson et al., 1987).

Herbicide Metabolism in the Recombinant Yeast

Metabolism of the various kinds of herbicide (chlortoluron, diuron, 2,4-D, bentazon, and chlorsulfuron) was determined in the culture of the recombinant yeast *Saccharomyces cerevisiae* strain AH22/pAFCR1 (Sakaki et al., 1994), producing the fused enzyme and AH22/pAAH5 (control). The yeast cells were grown to a density of 1×10^7 cells mL^{-1} in SD synthetic medium containing 2% (w/v) Glc, 0.67% (w/v) bact-yeast nitrogen base without amino acids (Difco, Detroit, MI), and 20 mg L^{-1} histidine, and each herbicide in acetone was added to a final concentration of 0.5 mM, except for 0.1 mM chlorsulfuron. Aliquots of culture supernatants acidified by 30% TCA were extracted twice with ethyl acetate and the organic phase recovered was evaporated to dryness at 30°C. Concentrated residues were dissolved in 20 μL of methanol. Extracts were analyzed by HPLC on a 4.6 × 150 mm COSMOSIL 5C18-AR column (Nacalai Tesque, Kyoto, Japan) with CH_3CN:aqueous 0.1% (w/v) sodium acetate (35:65, v/v). Chlortoluron and its metabolites were detected by A_{243}. The metabolites isolated by HPLC were each analyzed by MS using a Hitachi DF/GC/MS M-80B (Hitachi, Tokyo, Japan).

Vector / plasmid
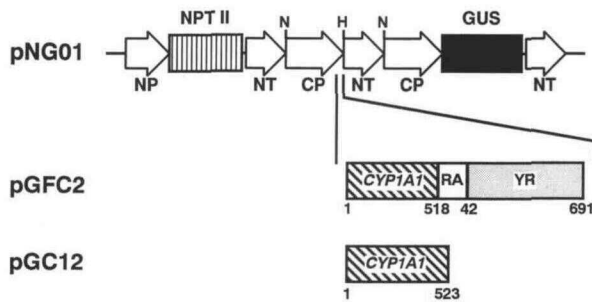
Figure 1. The structure of the constructed expression vector pNG01 and expression plasmids pGFC2 for the fused enzyme between rat CYP1A1 and yeast reductase and pGC12 for CYP1A1. *CYP1A1*, YR, NPT II, and GUS represent the coding regions for rat CYP1A1, yeast P450 reductase, neomycin phosphotransferase II, and GUS, respectively. NP, NT, and CP indicate Nos promoter, Nos terminator, and CaMV 35S promoter, respectively. R and A indicate amino acid residues derived from the synthesized DNA linker. N and H indicate the restriction sites of *Not*I and *Hind*III, respectively. Numbers below the coding regions indicate the number of amino acid residues as counted from the amino terminus of the corresponding enzymes.

Herbicide Resistance of the Transgenic Tobacco Plants

Transgenic tobacco plants (3–4 cm height) were grown on Murashige and Skoog medium containing chlortoluron at a concentration of 50 μM. The fresh weights of the whole plants were measured after 50 d.

RESULTS

Construction of Expression Plasmids

The plant expression vector pNG01 was constructed by replacement of the *Hind*III site of plasmid pBI121 with the *Not*I site, followed by insertion of the 1.1-kb *Not*I fragment containing the CaMV 35S promoter and Nos terminator derived from pBI221 into the *Not*I site. The plant expression plasmid pGFC2 for the fused enzyme between rat CYP1A1 and yeast reductase was constructed by insertion of the 3.5-kb *Hind*III fragment encoding the fused enzyme prepared from yeast expression plasmid pAFCR1 (Sakaki et al., 1994) into the unique *Hind*III site of pNG01. The fused enzyme contained a linker sequence Arg-Ala at the junction between the P450 and the reductase, which was optimized on the basis of the previous works. The expression plasmid pGC12 for CYP1A1 was also constructed by insertion of the 1.7-kb *Hind*III fragment encoding CYP1A1 alone prepared from pTF2 (Oeda et al., 1985) into the *Hind*III site of pNG01. Figure 1 schematically represents these constructed plasmids. The structure of the plasmids was confirmed by restriction mapping.
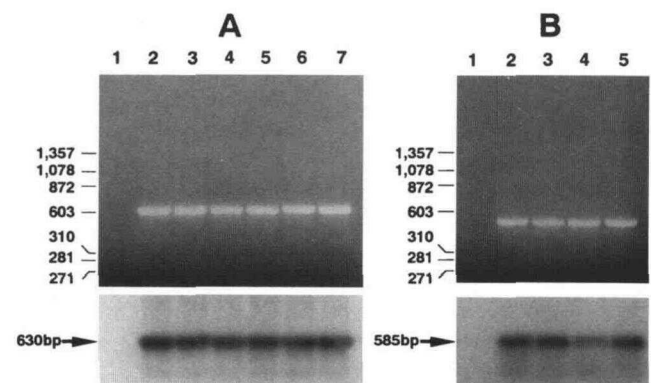
Transformation of Tobacco Plants

The expression plasmids pGFC2 and pGC12 were each mobilized into *A. tumefaciens* strain LBA4404 by triparental

mating procedures. Tobacco leaf discs were infected with *Agrobacterium* harboring each expression plasmid. Fifty-one regenerated tobacco plants transformed with the LBA4404/pGFC2 (the fused enzyme) and 39 plants transformed with LBA4404/pGC12 (CYP1A1) resistant to 200 mg L^{-1} kanamycin were initially selected approximately 50 d after *Agrobacterium* infection. These transformants were designated as GFC and GC plants, respectively, and assayed for GUS activity by histochemical and fluorescent procedures.

Genomic DNAs isolated from kanamycin-resistant, GUS-positive plants were analyzed for integration of the fused enzyme cDNA and CYP1A1 cDNA using PCR amplification. The 630-bp DNA fragment corresponding to the amino-terminal region and the 585-bp fragment corresponding to the carboxy-terminal region of the fused enzyme were both amplified in the genomic DNA of the GFC plants 3–1, 19–1, 22–2, and 31–1 (Fig. 2, upper). The 630-bp fragment corresponding to the amino-terminal region of CYP1A1 was also amplified in the GC plants 2–1 and 38–2. These PCR products were hybridized with the cDNA probes encoding the fused enzyme or CYP1A1 (Fig. 2, lower). No bands were amplified in the untransformed control plant. These results indicated that cDNAs for the fused enzyme and CYP1A1 were each integrated into the genomes of the selected plants.

Expression of CYP1A1 and Its Fused Enzyme in Tobacco Plants

GFC tobacco plants, in which the fused enzyme cDNA was integrated, were assayed for production of the fused enzyme protein by immunoblot analysis. The microsomal fractions prepared from the GFC plants 3–1, 19–1, 22–2, and 31–1 were analyzed by immunoblot using anti-CYP1A1 and anti-reductase antibodies. These GFC plants showed a 130-kD protein band reactive with both anti-CYP1A1 IgG and anti-reductase IgG, which migrated at the same position as the fused enzyme protein produced in the recombinant yeast

Figure 2. PCR detection of the fused enzyme and CYP1A1 cDNAs in the genomic DNA of the transgenic tobacco plants. Ethidium bromide-stained gels (upper) and blotted nylon membranes (lower) hybridized with the cDNA probes encoding the fused enzyme or CYP1A1. A, CYP1A1-specific primers; B, reductase-specific primers. Lane 1, Untransformed control plant; lanes 2 to 7, the transgenic plants GFC3–1, 19–1, 22–2, 31–1, GC2–1, and 38–2, respectively.
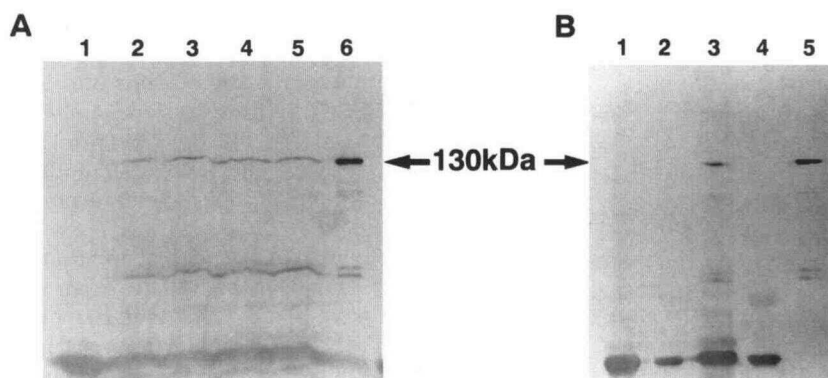
Figure 3. Immunoblot analysis of the fused enzyme protein produced in the GFC tobacco plants. A, Immunoblot of the SDS-solubilized microsomal proteins with anti-reductase antibody. Lane 1, Untransformed control plant (0.5 mg of protein); lanes 2 to 5, the transgenic plants GFC3–1, 19–1, 22–2, and 31–1, respectively (0.5 mg of protein); lane 6, the recombinant yeast AH22/pAFCR1. B, Immunoblot of the subcellular fractions prepared from untransformed control plant (lanes 1 and 2), GFC19–1 plant (lanes 3 and 4), and the recombinant yeast AH22/pAFCR1 (lane 5) with anti-yeast reductase antibody. Lanes 1, 3, and 5, Microsomal fractions (0.5 mg of protein); lanes 2 and 4, cytosol fractions (0.5 mg of protein). The arrows indicate the position of the fused enzyme protein.

AH22/pAFCR1 (Fig. 3A). The GC plants 2–1 and 38–2 also showed a 59-kD protein band reactive with anti-CYP1A1 IgG, which migrated the same position as CYP1A1 in the recombinant yeast AH22/pAMC1 (data not shown). These four GFC and two GC plants produced relatively higher amounts of the corresponding proteins among the selected transgenic plants. No identical bands were detected in the untransformed control plant. Immunoreactive bands with lower molecular masses probably represent products degraded during the extraction procedures.

Subcellular localization of the fused enzyme protein in GFC plants was determined by subjecting the microsomal and cytosol fractions prepared from the GFC19–1 plant to immunoblot analysis. A distinct band corresponding to the fused enzyme protein was given in the microsomal fraction, although no identical band was detected in the cytosol fraction (Fig. 3B). Similar results were obtained with the subcellular fractions prepared from the GC2–1 plant expressing CYP1A1 alone (data not shown). These results suggested that the fused enzyme and CYP1A1 expressed in the transgenic tobacco plants were localized primarily in the microsomal fraction.

Northern blot analysis of the total RNA prepared from the GFC19–1 plant revealed that an mRNA band hybridizing with the cDNA probe encoding the fused enzyme was found in leaves, stems, and roots (data not shown). The size of the band was consistent with that deduced from the cDNA structure. No identical band was detected in the control plant. The predicted size of an mRNA band for CYP1A1 was also detected in the total RNA from the GC2–1 plant (data not shown).

Monooxygenase Activity in the Transgenic Tobacco Plants

Monooxygenase activities toward 7-ethoxycoumarin and benzo[a]pyrene in the microsomal fractions of the transgenic tobacco plants were examined. The GFC plants 3–1, 19–1, 22–2, and 31–1 showed approximately 4 to 10 times higher

7-ethoxycoumarin O-deethylation activity than that of the control plant, whereas activities of the GC plants 2–1 and 38–2 were comparable to those in the control plant (Table I). The GFC plants also showed approximately 3 to 10 times higher benzo[a]pyrene 3-hydroxylation activity than that of the control plant (Table I). In the presence of anti-reductase Ig fraction, the 7-ethoxycoumarin O-deethylation activity was inhibited to 20% and CO inhibited this activity to 27% (Table II); preimmune Ig had minimal inhibitory effect. These results strongly suggested that the fused enzyme expressed in the GFC plants was functionally active in the microsomal fraction.

Herbicide Metabolism in the Recombinant Yeast

When 0.5 mM of the herbicide chlortoluron was added to the culture of the recombinant yeast AH22/pAFCR1 produc-

Table I. *Monooxygenase activities in the microsomal fraction prepared from the transgenic tobacco plants*

Monooxygenase activities toward 7-ethoxycoumarin and benzo[a]pyrene were measured as described in "Materials and Methods." Results are means of three individual measurements for control and GFC plants, and a single measurement for GC plants. NT, Not tested.

Plant Line	Product	
	7-Hydroxycoumarin	3-Hydroxybenzo[a]pyrene
	pmol min⁻¹ mg⁻¹ protein	
Control	3.5	0.3
GFC		
3-1	15.2	1.0
19-1	33.4	3.1
22-2	13.2	1.8
31-1	26.9	3.0
GC		
2-1	4.0	NT
38-2	4.7	NT

Table II. *Inhibition of 7-ethoxycoumarin O-deethylation activity in the microsomal fraction prepared from GFC19-1 plant*

7-Ethoxycoumarin O-deethylation activity was measured as described in "Materials and Methods" in the presence of inhibitors shown. Results are means of two individual measurements.

Inhibitor	Relative Activity
	%
None	100
Anti-yeast P450 reductase Ig	20
Preimmune Ig	92
CO	27

ing the fused enzyme, chlortoluron in the culture supernatant was decreased to 33% after 24 h of incubation, whereas no decrease was found in the control yeast AH22/pAAH5. HPLC analysis of the culture supernatant of AH22/pAFCR1 revealed the presence of three major metabolites derived from chlortoluron. Mass spectrometric analysis of these metabolites indicated that three metabolites correspond to ring-methyl-hydroxylated (M^+ 228), N-demethylated (M^+ 198), and ring-methyl hydroxy N-demethylated products (M^+ 214) (data not shown). These findings suggested that the metabolism of chlortoluron was catalyzed by the fused enzyme produced in the yeast AH22/pAFCR1 cells. The metabolism of other herbicides including diuron, 2,4-D, bentazon, and chlorsulfuron in the culture of the recombinant yeast was also examined. No metabolism was found in 2,4-D, bentazon, and chlorsulfuron, although diuron, which had a chemical structure similar to that of chlortoluron, was found to be metabolized (data not shown).

Herbicide Resistance of the Transgenic Tobacco Plants

Both GFC19–1 and untransformed control tobacco plants were grown on Murashige and Skoog medium containing chlortoluron at a concentration of 50 μM, one-tenth of the concentration used in the yeast culture. The GFC19–1 plant showed normal growth, whereas the control plant had damage with chlorosis of the leaves (Fig. 4). After 50 d of growth, the fresh weight of the whole GFC19–1 plant was 7.8 ± 0.9 g and the fresh weight of the control plant was 3.2 ± 1.1 g. Thus, the GFC19–1 plant expressing the fused enzyme was found to exhibit the resistance to the herbicide chlortoluron.

DISCUSSION

Rat CYP1A1 and its fused enzyme with yeast reductase were each expressed in the transgenic tobacco plants. Both CYP1A1 and the fused enzyme were localized primarily in the microsomal fraction of the plants. Saito et al. (1991) reported that rabbit liver CYP2C14 was expressed in transgenic tobacco plants. No P450-dependent monooxygenase activity was found, although some phenotypical changes and rapid senescence in the transgenic tobacco plants were observed. In the present study, the microsomal fraction prepared from the GFC plants expressing the fused enzyme showed enhanced monooxygenase activities toward both 7-ethoxycoumarin and benzo[a]pyrene compared with those of

the control plant, indicating that the fused enzyme was functionally expressed in the GFC plants. On the contrary, the expression of CYP1A1 alone seemed not to be enough for the enhancement of monooxygenase activities in the GC plants. Shet et al. (1993) reported that rat reductase was more effective than the purified mung bean reductase on monooxygenase activities in in vitro reconstitution experiments performed with porcine CYP17A expressed in *E. coli*. We have now demonstrated that highly efficient electron transport from the reductase part to the P450 part in the fused enzyme could lead to the functional expression of P450 monooxygenase in the transgenic tobacco plants.

Densitometric analysis of band intensity compared with that from known amounts of the authentic proteins expressed in the recombinant yeast strains indicated that the amounts of the fused enzyme and CYP1A1 produced in the transgenic tobacco plants were 10 pmol mg^{-1} protein or less. The expression levels of the fused enzyme and CYP1A1 produced in the yeast (Sakaki et al., 1994) were 13 and 21 times higher than those in the transgenic tobacco plants, respectively. With 7-ethoxycoumarin as a substrate, the turnover number of the fused enzyme in the microsomal fraction of the GFC19–1 plant was approximately 3 mol min^{-1} mol^{-1} P450, whereas it was 62 mol min^{-1} mol^{-1} P450 in the yeast (T. Sakaki, personal communication). These may be due to low substrate availability or improper folding of the fused enzyme expressed in the transgenic tobacco plants. Low heme incorporation into the synthesized Cyt protein may lead to low turnover number of the fused enzyme, as has been reported with the expression of P450 mediated by the baculovirus system (Asseffa et al., 1989).

It has been reported that the herbicide chlortoluron was metabolized to ring-methyl-hydroxylated and N-demethylated products in wheat (Mougin et al., 1990) and maize (Fonne-Pfister et al., 1990) plants by P450-dependent monooxygenases. P450-dependent metabolism in wheat and maize were important for the selective action of phenylurea

Figure 4. Herbicide resistance of GFC19–1 plant expressing the fused enzyme. Untransformed control (left) and GFC19–1 (right) plants were cultured for 50 d after they were transplanted onto Murashige and Skoog medium containing chlortoluron at the concentration of 50 μM.

herbicides. In wheat, ring-methyl hydroxylation and N-de-methylation of chlortoluron has been shown to be catalyzed by different P450 isoforms (Mougin et al., 1990, 1991). In the culture of the recombinant yeast AH22/pAFCR1 cells, chlortoluron was metabolized to ring-methyl-hydroxylated, N-demethylated, and ring-methyl hydroxy N-demethylated products. It is noteworthy that the fused enzyme catalyzes both ring-methyl hydroxylation and N-demethylation of chlortoluron. When the GFC19-1 plant expressing the fused enzyme grew on Murashige and Skoog medium containing chlortoluron, these ring-methyl-hydroxylated and N-demethylated metabolites were found in the plant extracts (N. Shiota and H. Ohkawa, unpublished data). The GFC19-1 plant showed a normal growth on Murashige and Skoog medium containing chlortoluron, whereas growth retardation was caused in the control plant. Thus, it was found that the fused enzyme expressed in the GFC plants conferred the resistance to chlortoluron.

Several transgenic plants resistant to herbicides have already been produced by introducing herbicide-metabolizing genes such as the phosphinothricin acetyltransferase gene (De Block et al., 1987; Thompson et al., 1987) and the bromoxynil nitrilase gene (Stalker et al., 1988), which were originated from bacteria. Romesser and O'Keefe (1986) and O'Keefe et al. (1988) showed that *Streptomyces griseolus* ATCC 11796 has two inducible P450 monooxygenases, P450SU1 and P450SU2 (CYP105A1 and CYP105B1), catalyzing sulfonylurea herbicide metabolism. These monooxygenase systems consisted of P450s, low molecular mass Fds, and NAD(P)H-Fd reductase. The fused enzyme between rat CYP1A1 and yeast reductase used in the present study was catalytically self-sufficient. In addition, mammalian drug-metabolizing P450 species have the catalytic versatility derived from the enzyme multiplicity as well as the overlapping substrate specificity of each species. These have a great potential for production of genetically engineered plants metabolizing a wide range of xenobiotics, including herbicides and environmental contaminants.

ACKNOWLEDGMENTS

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