Isosafrole-induced Cytochrome P_2-450 in DBA/2N Mouse Liver

CHARACTERIZATION AND GENETIC CONTROL OF INDUCTION*

Tohru Ohyama, Daniel W. Nebert, and Masahiko Negishif

From the Laboratory of Developmental Pharmacology, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, Maryland 20205

Mouse "cytochrome P_2-450" is defined as that form of isosafrole-induced P-450 in DBA/2N liver most specifically correlated with isosafrole metabolism. Isosafrole pretreatment does not induce aryl hydrocarbon hydroxylase activity ("cytochrome P_4-450") in C57BL/6N or DBA/2N mice, whereas isosafrole metabolism formation more than 3-fold in both C57BL/6N and DBA/2N mice. P_2-450 was, therefore, purified from isosafrole-treated DBA/2N liver microsomes having negligible amounts of contaminating P_1-450 and P_3-450. The apparent molecular weight of P_2-450 is 55,000, and the protein appears homogeneous on sodium dodecyl sulfate-polycrylamide gels. The Soret peak of the reduced purified cytochrome CO complex is 448 nm. Purified P_2-450, reconstituted in vitro, metabolizes acetophenone poorly and benzaldehyde hardly at all. Anti-(P_2-450) inhibits (90 to 100%) liver microsomal isosafrole metabolite formation, yet has no effect on aryl hydrocarbon hydroxylase, acetanilide 4-hydroxylase, or 7-ethoxycoumarin O-de-ethylase activities. 3-Methylcholanthrene induces anti-(P_2-450)-precipitable protein about 12-fold in C57BL/6N liver and 2-fold in DBA/2N liver; 2,3,7,8-tetrachlorodibenzo-p-dioxin (10 μg/kg), about 12-fold in both C57BL/6N and DBA/2N liver; isosafrole, more than 3-fold in both C57BL/6N and DBA/2N liver. Benzoylalanine at maximal dose induces anti-(P_2-450)-precipitable protein in C57BL/6N liver no more than 2-fold, yet is known to be a highly potent inducer of P_1-450 mRNA in C57BL/6N liver.

The sensitivity of the P_2-450 induction process to isosafrole is inherited as an autosomal additive trait; studies of offspring from the C57BL/6N(DBA/N)F_1 X DBA/2N backcross confirm involvement of the Ah locus or a closely segregating gene. In contrast, among crosses between C57BL/6N and DBA/2N, sensitivity of the P_1-450 and P_2-450 induction process to 3-methylcholanthrene or 2,3,7,8-tetrachlorodibenzo-p-dioxin is inherited as an autosomal dominant trait. These data suggest that, although P_1-450, P_2-450, and P_3-450 proteins are controlled by the Ah locus, either a P-450 protein polymorphism exists between C57BL/6N and DBA/2N mice or subtle differences may exist in the interaction of various inducers with Ah receptor.

The biochemical purification and characterization of a growing number of distinct cytochrome P-450 proteins has received increasing attention in recent years. More than 300 foreign chemicals, steroids, and peptide hormones are known to enhance P-450-mediated monooxygenase activities (reviewed in Refs. 4 and 5), suggesting the induction of one or more P-450 proteins in each instance, and the list grows larger each year.

To understand the genetic mechanisms by which a particular chemical is able to induce a particular P-450 protein has been the central goal of this laboratory. Most of our work has involved the expression of mouse P-450, controlled by the Ah receptor (6-8). These studies have always taken advantage of the fact that the B6 mouse (Ah<sup>b</sup>/Ah<sup>b</sup>) has a large amount of the high affinity receptor and the D2 mouse (Ah<sup>b</sup>/Ah<sup>′</sup>) has a poor affinity receptor. Following 3-methylcholanthrene treatment, P_1-450 is thus highly induced in B6 mouse liver and negligibly induced in D2 mouse liver. P_4-450 induction by 3-methylcholanthrene is inherited in the B6D2F<sub>1</sub>; heterozygote (Ah<sup>b</sup>/Ah<sup>′</sup>) as an autosomal dominant trait (9).

Isosafrole is a naturally occurring plant constituent (10). Studying Ah<sup>b</sup>/Ah<sup>b</sup> and Ah<sup>′</sup>/Ah<sup>′</sup> mice, Fennell et al. (11) concluded that isosafrole induces a form of P-450 specific for

* Portions of this work were presented at the Fourth International Conference on Cytochrome P-450, Biochemistry, Biophysics and Environmental Implications, Kuopio, Finland, June, 1982 (1). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
1 Present address, Hokkaido Institute of Public Health, North 19, West 12, Sapporo, Hokkaido, Japan.
2 To whom correspondence should be sent.
3 Present address, Building 19, Room 19-10, National Institutes of Health, P.O. Box 12233, Research Triangle Park, NC 27709.

1 The term "P-450" is used to designate any or all forms of microsomal cytochrome P-450. Mouse "P-450" is defined as that form of isosafrole-induced P-450 in DBA/2N liver most specifically associated with isosafrole metabolism. Mouse "P-450" and "P-450" are defined as those forms of 3-methylcholanthrene-induced P-450 most specifically associated with induced aryl hydrocarbon hydroxylase and acetanilide 4-hydroxylase activities, respectively. The concentration of P_3-450 protein in control mouse microsomes is at least five times greater than that of P_1-450; after 3-methylcholanthrene induction of C57BL/6N liver, P_2-450 is the major induced form, being about five times greater in concentration than P_1-450. The size of all three proteins is 55,000 daltons, as judged by NaDodSO<sub>4</sub>-polyacrylamide gel electrophoresis. The Soret peaks of the reduced hemeprotein-CO complex for P_2-450, P_3-450, and P_4-450 are 448, 448, and 448 nm, respectively. Because we have now characterized two proteins with a spectral maximum of 448 nm, we have changed the name of "P-448" studied 5 years ago (3) to "P-450." Other abbreviations used are: isosafrole, 4-propenyl-1,2-methylenedioxybenzene; B<sub>6</sub>, the C57BL/6N inbred mouse strain; D2, the DBA/2N inbred mouse strain; ellipticine, 5,11-dimethyl-6H-pyridol[4,3-b]carbazole; p,p'-DDE, 2,2-bis(p-chlorophenyl)-1,1,1-trichloroethane; p,p'-DDE, 2,2-bis(p-chlorophenyl)-1,1-dichloroethylene; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; GED, buffer containing 20% glycerol, 1 mM ethylenediaminetetraacetic acid, and 1 mM dithiothreitol; NaDodSO<sub>4</sub>, sodium dodecyl sulfate; zoxazolamine, 2-amino-5-chlorobenzoxazole.
isosafrole metabolism and that this form is not identical to the major phenobarbital- or 3-methylcholanthrene-inducible forms of P-450. Subsequently, isosafrole-induced P-450 was purified from rat liver microsomes (12, 13). From these studies it was apparent that this isosafrole-induced P-450 is similar in many ways to the major rat 3-methylcholanthrene-inducible P-450 yet did not metabolize benzo[a]pyrene. These valuable studies (3, 6-8, 12, 13) were each performed with an antibody that was not claimed to be monospecific; despite this shortcoming many important conclusions have been made.

Whereas 3-methylcholanthrene induces P-450 in B6 but not D2 liver, isosafrole induces a form of P-450 almost equally well in both B6 and D2 liver (1, 11). In this report we show the isolation of the isosafrole-induced form (P-450) from isosafrole-treated D2 mouse liver microsomes, in which there is virtually no contaminating P-450. Further, we demonstrate that the P-450 induction by isosafrole is controlled by the Ah receptor but that the inheritance of this genetic trait is somewhat different from inheritance of the trait of P-450 induction by 2-methylcholanthrene.

**EXPERIMENTAL PROCEDURES**

**Materials**—Benzo[a]pyrene, 3-methylcholanthrene, benzo[a]anthracene, diethiothreitol, dilauroylphosphatidylethanolamine, 7-ethoxycoumarin, dexamethasone, NADPH, and NADH were purchased from Sigma; 2-hydroxybiphenyl, 4-hydroxybiphenyl, 2-methylbenzimidazole, acetanilide, and 4-hydroxyacetanilide from Aldrich; isosafrole from Eastman Kodak Company (Rochester, NY); sodium cholate and SB4 (a detergent, Tablegen TM8; N-tetracetyl-N,N-dimethyl-3-ammonio-1-propane sulfonate) from Calbiochem-Behring; hydroxylapatite (Bio-Gel HT) from Bio-Rad; sodium phenobarbital from Merck; NaB[3H]4 (7.0 Ci/mmol) from Amersham Corp.; acetanilide deuterated in our animal facility. Mazola corn oil was purchased from General Foods and progeny from B6D2Fl backcross to D2 were obtained from the National Institutes of Health Veterinary Resources Branch (Bethesda, MD). B6D2Fl offspring and progeny from B6D2Fl backcross to D2 were obtained from the National Institutes of Health Veterinary Resources Branch (Bethesda, MD). MD). Sources of other reagents have been cited previously (3, 15).

**Purification of Cytochrome P-450**—Liver microsomes prepared from 50 isosafrole-treated D2 mice were solubilized by 0.6% cholate in 200 mM potassium phosphate buffer-GED (pH 7.25) and centrifuged at 35,000 rpm for 90 min. The resultant supernatant was applied to an octyladimine-Sepharose 4B column (2.5 x 40 cm) equilibrated with 200 mM potassium phosphate buffer-GED (pH 7.25). After being washed with 100 mM potassium phosphate buffer-GED (pH 7.25), the P-450 was eluted with 100 mM potassium phosphate buffer-GED containing 0.05% Emulgen 913 and then with the same buffer containing 0.2% Emulgen 913 (Fig. 1). The fractions that were eluted with the 100 mM potassium phosphate buffer containing 0.2% Emulgen exhibited high specific P-450 content, and NaDodSO4-polyacrylamide gel electrophoresis (Fig. 1, inset) shows a relatively homogeneous preparation of cytochrome P-450, especially in fraction number 60. Fractions 55 to 65 were, therefore, pooled and diluted 4-fold with 28% glycerol, 1 mM dithiothreitol, and 0.2% Emulgen 913 and applied to an hydroxylapatite column equilibrated with 25 mM potassium phosphate buffer-GED (pH 7.25) plus 0.2% Emulgen 913. After being washed with 25 mM potassium phosphate buffer-GED in 0.2% Emulgen 913, P-450 was eluted with 50 mM potassium phosphate buffer-GED in 0.2% Emulgen 913. About 2 mg of P-450 (specific activity of -15.0 mmol/mg of protein) was obtained from 50 mice.

**Development of Anti-(P-450)**—A mixture of purified P-450 (500 μg for each dose, with complete Freund's adjuvant the first time, and incomplete Freund's adjuvant the next two times) was injected three times, at 2-week intervals, into a goat. Preimmune and postimmune immunoglobulin G fractions were prepared by ammonium sulfate fractionation. After dialysis the fractions were dissolved in 100 mM potassium phosphate buffer (pH 7.5). These fractions were used for inhibition of microsomal monooxygenase activities in vitro (3) and determination of titrated anti-(P-450)-precipitable protein from cholate-solubilized microsomal membranes that had been previously labeled with NaB[3H]4 in the presence of 1 mM pyridoxal phosphate (3, 20), by the methods cited. The values of anti-(P-450)-precipitable material are expressed as: (disintegrations min⁻¹ per mg of microsomal protein)/(disintegrations min⁻¹ per mg of total microsomal protein).

**Spectrophotometry**—Total P-450 content was determined by the method of Omura and Sato (21); an extinction coefficient of 91 mM⁻¹ cm⁻¹ was used for the difference in absorption between the Soret peak around 450 nm and the base-line at 490 nm for the dithionite-reduced hemoprotein. CO complex. The isosafrole metabolite cytochrome P-450 complex was assayed (13, 22) with an extinction coefficient of 75 mM⁻¹ cm⁻¹ for the difference in absorption between 455 nm and the base-line at 490 nm for the dithionite-reduced form (5). Protein concentrations were determined by the method of Lowry et al. (23), as modified by Dulley and Grieve (24) to eliminate interference by detergents.

**Enzyme Assays**—The determination of isosafrole metabolite formation (13, 22), aryl hydrocarbon hydroxylase (25), acetanilide 4-hydroxylase (26), biphenyl 2- and 4-hydroxylase (27), and 7-ethoxy-
olite bound to the reduced P-450 (Fe

protein was purified from isosafrole-treated D2 liver microsomes. The Soret peak of the reduced cytochrome CO complex was 448 nm. The apparent molecular weight was 55,000 (Fig. 3). By means of Ouchterlony double diffusion plates, fusion of a major precipitin line indicates immunologic homology of the anti-(P2-450)-precipitable protein in isosafrole-treated B6 and D2 control liver microsomes. An increased intensity of the precipitin line following isosafrole treatment is consistent with relatively equal induction of anti-

coumarin O-de-ethylase (28) activities were carried out by the procedures cited. Reconstitution experiments were performed with 30 µg of dilauroylphosphatidylcholine, 0.5 unit of NADPH P-450 reductase, and 100 pmol of P2-450 incubated at room temperature for 10 min; then the regular buffer and assay conditions for each activity were used, according to the previously published techniques.

Ah Receptor Assay—The postmicrosomal supernatant of individual livers from untreated mice was exposed to 10 nM [3H]TCDD (in the absence or presence of nonlabeled 1 µM TCDD) for 1 h at 4 °C. Following dextran-charcoal adsorption, the material was centrifuged on a linear (5 to 20%) sucrose density gradient, as detailed (29). The saturable radioactivity in the Ah receptor peak was equated with femtomoles of receptor per mg of cytosolic protein and with the number of TCDD-binding sites per cell (29).

RESULTS

The P2-450 Protein—As detailed under "Experimental Procedures" and in Fig. 1, an electrophoretically homogeneous protein was purified from isosafrole-treated D2 liver microsomes. The Soret peak of the reduced cytochrome-CO complex was 449 nm in microsomes (Fig. 2), but the Soret peak of the purified P2-450-CO complex was 448 nm. The apparent molecular weight was 55,000 (Fig. 3). By means of Ouchterlony double diffusion plates, fusion of a major precipitin line between neighboring wells (Fig. 4) indicates immunologic homology of the anti-(P2-450)-precipitable protein in isosafrole-treated B6 and D2 control liver microsomes. An increased intensity of the precipitin line following isosafrole treatment is consistent with relatively equal induction of anti-(P2-450)-precipitable material in both D2 and B6 mice. A possible minor precipitin line nearer to well 5 than the major line (Fig. 4) suggests that the antisera may recognize at least a second microsomal protein; we are not claiming, however, that anti-(P2-450) is monospecific. There are clearly no spurs seen. Between the P2-450 and P2-450 proteins (data not shown), there is no fusing of precipitin lines indicating that anti-(P2-450) does not cross-react with P1-450.

Association of P2-450 with Isosafrole Metabolism—Whereas isosafrole did not induce aryl hydrocarbon hydroxylase activity in the intact liver microsomes of D2 and B6 mice (Table I), isosafrole did induce acetanilide 4-hydroxylase activity about 3-fold in B6 but not at all in D2 mice. These data indicate that isosafrole is not a good inducer of P2-450 in either D2 or B6 mice and does not induce P2-450 in D2 mice.
3-Methylcholanthrene, on the other hand, induced both aryl hydrocarbon hydroxylase and acetanilide 4-hydroxylase activity more than 6-fold in B6 mice and not at all in D2 mice. These data have been reported previously (3, 27).

The P₂-450-reconstituted activities of aryl hydrocarbon hydroxylase and acetanilide 4-hydroxylase (Table I) are about one-thirtieth and one-tenth, respectively, of these activities in 3-methylcholanthrene-induced B6 liver intact microsomes. The rate of benzo[a]pyrene metabolism by purified P₁-450 reconstituted in vitro (3) was 2.95 nmol/min/nmol of P-450, as compared with the P₂-450 value of 0.14 in Table I. The rate of acetanilide 4-hydroxylation by purified P₂-450 reconstituted in vitro (3) was 32 nmol/min/nmol of P-450, as compared with the P₂-450 value of 1.52 in Table I. These findings suggest that, whereas there exist overlapping substrate specificities, neither benzo[a]pyrene nor acetanilide is a particularly good substrate for P₂-450. Further, the results in Table I show that isosafrole-induced P₂-450 in D₂ mice is different from 3-methylcholanthrene-induced P₁-450 or P₂-450 in B6 mice.

Isosafrole induced total P₄-450 content in D₂ liver (Table II), whereas 3-methylcholanthrene had no effect in D₂ liver. In B6 liver, 3-methylcholanthrene induced total P₄-450 content better than isosafrole treatment. It is known (12, 13, 22) that the metabolite forms a complex with the induced protein.

**Table I**

<table>
<thead>
<tr>
<th>Aryl hydrocarbon hydroxylase and acetanilide 4-hydroxylase activity in intact liver microsomes and with purified P₂-450 reconstituted in vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Intact microsomes</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Reconstituted</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

**Table II**

<table>
<thead>
<tr>
<th>Formation of the P₂-450 complex with isosafrole metabolite and the effect of anti-(P₂-450) on this formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inbred strain</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>D₂</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>B6</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

The formation of this complex can be measured by following the increase of absorption at 456 nm (Fig. 2). The amount of isosafrole metabolite bound to P₂-450 in vivo was about the same as that bound in vitro following incubation of microsomes with substrate and NADPH at 37 °C.

Anti-(P₂-450) inhibited isosafrole metabolite formation completely in isosafrole-treated D₂ mice and more than 90% in isosafrole-treated B₆ mice (Table II). The antibody had little effect on isosafrole metabolite formation in 3-methylcholanthrene-treated D₂ mice and blocked more than half of the activity in 3-methylcholanthrene-treated B₆ mice. Anti-(P₂-450) had no appreciable effect on isosafrole metabolite formation in control or phenobarbital-treated B₆ or D₂ mice. Fig. 5 illustrates that anti-(P₂-450) is a good inhibitor of isosafrole activity but does not inhibit aryl hydrocarbon hydroxylase, acetanilide 4-hydroxylase, biphenyl 2- or 4-hydroxylase, or 7-ethoxycoumarin O-de-ethylase activities in isosafrole-treated or 3-methylcholanthrene-treated B₂ and D₂ mice. These findings lend further support to the concept that P₂-450 is distinct from both mouse P₁-450 and P₃-450.
FIG. 5. Effect of anti-(P2-450) on six liver microsomal monooxygenase activities. Liver microsomes from five mice were pooled for each group, and at least two experiments were performed in duplicate for each value shown. Inducer treatment included 3-methylcholanthrene (MeChol) and isosafrole. Liver microsomes from isosafrole-treated B6 and D2 were examined as freshly prepared intact samples (Isosafrole) and also after the incubation of microsomes with 17 mM 2-methylbenzimidazole (15 min at 37 °C); this experiment (13, 22) was designed to displace any isosafrole metabolite that might interfere with any one of the monooxygenase activities (Isosafrole-displaced). Further details are described under “Experimental Procedures.”

P2-450 almost as well. These data are consistent with the finding (30) that D2 mice have a poor affinity Ah receptor that can be overwhelmed by sufficient amounts of TCDD; when this occurs, the TCDD-receptor complex enters the nucleus and elicits its effect of P2-450 mRNA induction (30). The results with 3-methylcholanthrene and TCDD in B6 and D2 mice in Fig. 7 thus suggest that induction of the anti-(P2-450)-precipitable material might be controlled by the Ah receptor (5, 6).

Benzo[a]anthracene induced the immunoprecipitate only about 2-fold in B6 and negligibly in D2 mice (Fig. 7). It is known (17) that the dose of benzo[a]anthracene used induces P2-450 mRNA very effectively in B6 but not D2 mice. Ellipticine behaved like isosafrole by inducing anti-(P2-450)-precipitable protein equally well in B6 and D2 mice.2 Pregnenolone 16α-carbonitrile, dexamethasone, p,p'-DDE, Dicofol, and chlorobenzylate; the results were not significantly different from that seen with p,p'-DDT, dexamethasone, pregnenolone 16α-carbonitrile, or phenobarbital treatment. The amount of immunoprecipitated P2-450 protein labeled with tritium was calculated on the basis of disintegrations per min per μg of protein in the cholate-solubilized fraction. Individual samples varied less than 10%. Further experimental details are described under “Experimental Procedures,” the legend to Fig. 6, and in Refs. 3 and 20.

2 Isosafrole, benzo[a]anthracene, and ellipticine displaces 67, 61, and 40%, respectively, of [3H]TCDD from the Ah receptor (31), when these test compounds are present in 100-fold excess, compared with the radioligand concentration of 10 nM; in the same experiments, 3-methylcholanthrene and nonlabeled TCDD displace the radioligand 95 and 100%, respectively (31).
isopropylemic P2-450 in D2 Mouse

Induction of Anti-(P2-450)-precipitable Material by Isosafrole in B6, D2, and B6D2F1 Mice—Although isosafrole at 150 mg/kg induces the immunoprecipitable equally well in B6 and D2 mice, smaller doses exhibited a differential effect, with B6 being about 10 times more sensitive than D2 (Fig. 8). Moreover, the B6D2F1 heterozygote demonstrated a response exactly midway between the B6 and D2 parents. No difference between males and females was seen. This type of dose-response curve is thus consistent with autosomal additive inheritance. Among B6, D2, and B6D2F1 mice, the trait of UDP-glucuronosyltransferase induction by 3-methylcholanthrene is inherited additively (32), whereas the trait of aryl hydrocarbon hydroxylase (P2-450) induction by 3-methylcholanthrene (33) or acetanilide 4-hydroxylase (P3-450) induction by 3-methylcholanthrene (27) is autosomal dominant. Dose-response curves with TCDD (32, 34) also have demonstrated that UDP-glucuronosyltransferase induction is inherited additively (i.e. B6D2F1 is intermediate between B6 and D2) while P2-450 induction is inherited dominantly (i.e. B6D2F1 is not statistically different from B6).

Association of the Ah Locus with Induction of the Immunoprecipitable by Isosafrole—An intermediate dose of isosafrole (15 mg/kg) was used to demonstrate that the additive inheritance of P2-450 induction follows Mendelian genetics and correlates well with the Ah phenotype (Fig. 9). The B6D2F1 was intermediate between the B6 and D2 parents. From offspring of the B6D2F1 x B6 backcross, about one-half appeared to be similar to B6 and about one-half similar to the F1. From offspring of the B6D2F1 x D2 backcross, about one-half appeared to be similar to the F1, and about one-half similar to the D2 parent. Although these individual mouse values are consistent with single-gene inheritance of an additive trait, there is sufficient scatter of points to suggest more than a single gene being involved.

In Fig. 9 (far right), eleven offspring from the B6D2F1 x D2 backcross were Ah-phenotyped by the zoxazolamine paralysis test (35) 2 weeks previously and then their liver microsomes were examined for anti-(P2-450)-precipitable protein.

3 Because aryl hydrocarbon hydroxylase activity is not induced by isosafrole, we cannot conveniently study aryl hydrocarbon hydroxylase induction in this experiment.

Whereas five Ah+/Ahb and all five Ahb/Ah− mice behaved as expected, one Ahb/Ah− mouse appeared to be a recombinant. The data in Fig. 9 provide genetic proof that P2-450 induction by isosafrole is associated with the Ah locus or a closely segregating gene. The fact that one recombinant occurred out of eleven backcross offspring examined (Fig. 9) suggest that there exists some possible crossover event, or less than perfect association, between P2-450 induction by isosafrole and Ah-phenotyping by the zoxazolamine paralysis test.

Association of Ah Receptor with P2-450 Induction by Isosafrole—Fig. 10 is an illustration of the difference in response among B6, D2, and B6D2F1 mice between induction of anti-(P2-450)-precipitable protein by isosafrole and P2-450 induction by 3-methylcholanthrene. The former trait is inherited additively, the latter, dominantly. The results in Figs. 8, 9, and 10 thus demonstrate the involvement of the Ah receptor but indicate some slightly different manner in which the inducer-receptor complex effects its response: P2-450 induction being like UDP glucuronosyltransferase induction and different from P1-450 or P3-450 induction.

DISCUSSION

In this report we have shown the isolation and characterization of a new mouse P-450 protein called P2-450. We have demonstrated that P2-450 is unique via studies of catalytic activity, antibody inhibition of catalytic activities, spectral data, response of the immunoprecipitated protein to various classical P-450 inducers, and genetic expression of its induction among the appropriate crosses between B6 and D2 mice. An antibody's "lack of inhibition of catalytic activity" need not always be correlated with the antibody's "lack of immu-
mice. P-Naphthoflavone (37) presumably belongs to this possibility of doses, induces P1-450 in B6 but negligibly in D2 mice (30, 36). TCDD also has the same effect on P2-450 (Fig. 7B) and P3-450 (2) induction. Third, isosafrole at low doses induces the anti-(P2-450)-precipitable protein in B6 but not D2 mice and at high doses induces the immunoprecipitate equally well in both B6 and D2 mice. However, isosafrole at high doses does not induce P1-450 and induces acetanilide 4-hydroxylase activity in B6 but not D2 mice. Fourth, benzo[a]anthracene is an excellent inducer of P1-450 mRNA in B6 but not D2 mice (17) but induces the P2-450 immunoprecipitate very poorly in B6 mice.

In terms of classical receptor studies, when an inducer-receptor complex forms and interacts with an intranuclear target, one specific response is elicited. If a different response is elicited with a second inducer, this suggests a distinctly different inducer-receptor complex. Therefore, it seems impossible that any two of the above-mentioned four classes of compounds are acting precisely in the same manner. One possible explanation is that the Ah receptor population is heterogeneous (31). The other possibility is that a single type of Ah receptor molecule exists but that each of several inducers hysteretically produces a somewhat different structure of the inducer-receptor complex, thereby exerting different signals during the activation of the three P-450 genes, plus one or more UDP-glucuronosyltransferase(s). Further studies with these four (or more) proteins, and especially their corresponding genes, should shed new light on this perplexing problem.

Figure 10. Amount of anti-(P2-450)-precipitable (top) and anti-(P3-450)-precipitable (bottom) protein from cholate-solubilized microsomes, as a function of Ah receptor levels, in livers of B6, D2, and B6D2F1 mice. The experimental protocol was the same as that in Figs. 7 and 8. The anti-(P-450) used here has been well characterized (3). Groups of five mice were determined individually; symbols and brackets denote means ± S.D. Top, mice received isosafrole (15 mg/kg) for each of three consecutive days and were killed 24 h after the last dose. Bottom, mice received 3-methylcholanthrene (200 mg/kg) as a single intraperitoneal dose 3 days before killing. Ah receptor determinations (29) were carried out on five individual mice per group, and these could not be the same mice as those treated with isosafrole or 3-methylcholanthrene (because prior treatment with inducer in vivo saturates Ah receptor-binding sites, thereby making the in vitro receptor assay invalid (29)).

noprecipitation." It is also possible that an antibody might precipitate the protein without blocking its catalytic activity. P2-450 seems highly specific for isosafrole metabolite formation and metabolizes acetanilide and benzo[a]pyrene very poorly. Anti-P2-450 blocks the isosafrole metabolite formation virtually 100% and has no effect on acetanilide 4-hydroxylation, benzo[a]pyrene hydroxylation, biphenyl 2- or 4-hydroxylation, or 3-ethoxyxoumarin O-de-ethylation.

The additive inheritance of the induction of anti-(P2-450)-precipitable protein between B6 and D2 mice might be explained on the basis of a P-450 polymorphism between the two inbred strains. Complete nucleotide sequencing of the mRNA from both strains will rule this possibility in or out.

The variable types of inheritance and "induction responses" of P1-450, P2-450, and P3-450 by several polycyclic aromatic compounds may be difficult to resolve by postulating a single species of Ah receptor. It, therefore, appears that there exist at least four groups of polycyclic aromatic compounds which interact in slightly different ways with the Ah receptor to induce P1-450, P2-450, and P3-450 differentially. First, 3-methylcholanthrene, when given at the highest experimentally possible doses, induces P1-450 in B6 but negligibly in D2 mice (36). 3-Methylcholanthrene also induces the P2-450 immunoprecipitate and P2-450 very well in B6 mice but not D2 mice. β-Naphthoflavone (57) presumably belongs to this 3-methylcholanthrene class. Second, TCDD at low doses induces P1-450 in B6 but not D2 mice yet at high doses induces P1-450 equally well in both B6 and D2 mice (30, 36). TCDD also has the same effect on P2-450 (Fig. 7B) and P3-450 (2) induction. Third, isosafrole at low doses induces the anti-(P2-450)-precipitable protein in B6 but not D2 mice and at high doses induces the immunoprecipitate equally well in both B6 and D2 mice.

**REFERENCES**


Isosafrole-induced P450 in D2 Mouse

Isosafrole-induced cytochrome P2-450 in DBA/2N mouse liver. Characterization and genetic control of induction.
T Ohyama, D W Nebert and M Negishi


Access the most updated version of this article at http://www.jbc.org/content/259/4/2675

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/259/4/2675.full.html#ref-list-1