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A NOVEL FORM OF CYTOCHROME P-450 IN BEAGLE DOGS

P-450-D3 IS A LOW SPIN FORM OF CYTOCHROME P-450 BUT WITH CATALYTIC AND STRUCTURAL PROPERTIES SIMILAR TO P-450d

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Abstract—A form of cytochrome P-450, P-450-D3, cross reactive with antibodies to rat P-450d was purified from liver microsomes of polychlorinated biphenyl (PCB)-treated female Beagle dogs to an electrophoretic homogeneity. Judging from the result of sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE), the molecular weight of P-450-D3 was estimated to be 54,000. The oxidized form of P-450-D3 showed a peak at 416 nm indicating that the cytochrome is mostly in a low spin state. The carbon monoxide bound reduced form of P-450-D3 showed a peak at 448 nm. In a reconstituted system, P-450-D3 catalyzed drug oxidations including benzphetamine and aminopyrine *N*-demethylations, 7-ethoxycoumarin and *p*-propoxyaniline *O*-dealkylations, and aniline and benzo(*a*)pyrene hydroxylations. The rate of aniline hydroxylation catalyzed by P-450-D3 was similar to that catalyzed by P-450c which is a low spin form of cytochrome P-450 purified from liver microsomes of PCB-treated rats, whereas the catalytic activities of P-450-D3 for 7-ethoxycoumarin *O*-deethylation and benzo(*a*)pyrene hydroxylation were considerably lower than those of P-450c. The amino terminal portion of P-450-D3 was found to be highly similar to those of P-450d, human P_3-450 and P_3-450 when four amino acid deletions were tentatively inserted between fifth and sixth amino acids from the *N*-terminal, but not that of P-450c which is a low spin form of cytochrome P-448 purified from rat liver microsomes. These results indicate that Beagle dogs possess a low spin form of cytochrome P-450 with spectral properties similar to P-450c but with catalytic and structural properties similar to P-450d.

As one of the characteristics of hepatic microsomal monooxygenase system, it should be pointed out that pretreatment of animals with drugs and chemicals induce a specific form(s) of cytochrome P-450 depending on drugs administered [1, 2]. A number of different forms of cytochrome P-450 have been purified from liver microsomes of untreated and drug-treated animals, and characterized in detail. However, the data on the properties of cytochrome P-450 in dog liver microsomes [3, 4] are limited compared to those in other animal species including rats, rabbits and mice. It was known that the inducers can be primarily divided into two groups, one of which is typified by phenobarbital which preferentially induces P-450b and P-450e, and the other typified by 3-methylcholanthrene which preferentially induces P-450c and P-450d [5]. The toxicological significance of P-450c and P-450d, identical with our PCB P-448-L and PCB P-448-H [6], have been extensively studied because of their ability to activate a wide variety of promutagens and carcinogens. The existence of forms of cytochrome P-450 immunochemically, structurally and catalytically related P-450c and P-450d among animal species including mice and humans have been proposed, while the cytochrome in Beagle dogs which are used in preclinical studies in laboratories have not been investigated as yet.

In a previous paper, we reported on the properties of a high spin form of cytochrome P-450 (P-450-D2*) from liver microsomes of PCB-treated Beagle dogs [8] which is immunochemically and functionally analogous to rat P-450d. We report herein on a novel form of Beagle dog cytochrome P-450, tentatively designated as P-450-D3, which shows spectral properties similar to P-450c but catalytic and structural properties similar to P-450d.

MATERIALS AND METHODS

Materials. Salmonella typhimurium (TA 98 and TA 100), benzphetamine and Emulgen 911 were kindly provided by Dr. Uematsu of Hokkaido Pharmaceutical College, Upjohn Co., and Kao Atlas Co., respectively. 3-Amino-1-methyl-5H-pyrido(4,3-b)-indole acetate (Trp-P-2) and 2-amino-6-methyldipyridol(1,2-a:3′,2′-d)imidazole acetate (Glu-P-1) were kindly donated by Drs. T. Sugimura and M. Nagao, National Cancer Center Institute, Tokyo. NADP, NADPH, glucose 6-phosphate and glucose 6-phosphate dehydrogenase were purchased from Oriental Yeast, Tokyo, Japan, and dilauroyl-L-3-phosphatidylcholine was from Wako Pure Chemical Co., Tokyo, Japan. All other reagents were of the highest purity commercially available.

Animals. Adult female Beagle dogs weighing

* According to the newly-recommended nomenclature for P-450 enzymes [7], both P-450-D2 and P-450-D3 are the cytochrome P-450 which can be probably classified into P-450 I family.

about 10 kg and male Sprague–Dawley rats (8–11 weeks) were used for purification experiments. Animals were given a single intraperitoneal injection of PCB (Kaneclor KC-500) in corn oil at a dose of 400 mg/kg and killed 5 days later.

Purification of P-450-D3. Dog liver microsomes were prepared as described previously [9] and were solubilized with sodium cholate [10]. Solubilized supernatant was applied onto an ω-aminooctyl Sepharose 4B column (2.6 × 57 cm) which had been equilibrated with 100 mM potassium–phosphate buffer (pH 7.4) containing 20% glycerol, 1 mM DTT, 0.1 mM EDTA and 0.5% sodium cholate (Buffer A). The column was washed with a column volume of 10 mM potassium–phosphate buffer (pH 7.4) containing other components as buffer A (Buffer B), and then with 500 ml of buffer B containing 0.2% Emulgen 911. Cytochrome P-450 was eluted by washing the column with linear gradients of the concentrations of potassium phosphate (10–200 mM) and Emulgen 911 (0.2–0.5%) in a total volume of 1000 ml. The eluate was monitored for cytochrome P-450 with absorbance at 417 nm. Then, the fractions containing cytochrome P-450 immunoreactive with crude anti-P-450d antibodies were pooled and were concentrated by ultrafiltration on a UK-50 membrane (Toyo Roshi, Tokyo, Japan). Further purification was conducted by means of a high performance liquid chromatography (HPLC) equipped with a preparative DEAE-5PW column (Toyo Soda, Tokyo, Japan). The concentrated pooled fraction was diluted 10-fold with 20 mM Tris–acetate buffer (pH 7.5) containing 20% glycerol, and was applied onto a preparative DEAE-5PW column which had been equilibrated with 20 mM Tris–acetate buffer (pH 7.5) containing 20% glycerol and 0.4% Emulgen 911. After the column was washed with about 30 ml of equilibrating buffer, cytochrome P-450 was eluted by washing the column with a linear gradient of the concentration of sodium acetate as described by Funae and Imaoka [11].

Reconstitution of monooxygenase system. A typical incubation mixture contained 100 mM potassium phosphate buffer (pH 7.4), 50 pmole of P-450-D3, 0.5 unit of rat NADPH-cytochrome P-450 reductase, 25 μg of dilauroyl-L-3-phosphatidylcholine, 0.1 mM EDTA, an NADPH-generating system (0.5 mM NADP, 5 mM glucose 6-phosphate, 1 unit of glucose 6-phosphate dehydrogenase and 5 mM $MgCl_2$) and a substrate in a final volume of 0.5 ml. All assays were carried out with shaking at 37° aerobically for 15 min. Product formation in each reaction was linear with time under these conditions. *N*-Demethylase activities of benzphetamine and aminopyrine were estimated by determination of formaldehyde according to the method of Nash [12]. Activities of 7-ethoxycoumarin *O*-deethylase and benzo(*a*)pyrene hydroxylase were estimated by the method of Aitio [13] and Nebert and Gelboin [14], respectively. Aniline hydroxylase and *p*-propoxyaniline *O*-depropylase activities were estimated by determination of *p*-aminophenol according to the method of Imai *et al.* [15].

Mutation assay. Salmonella typhimurium TA98 or TA100 was grown in 0.5% sodium chloride-containing neutrient broth to the log phase. The incubation mixture contained components essentially the same for the assay of monooxygenase activities except that bacteria cells, promutagens and 0.1 nmole of P-450-D3 were added to the incubation mixture. Aflatoxin B_1 and benzo(*a*)pyrene were dissolved in dimethylsulfoxide (DMSO) and 10 μl of the DMSO solution was added to each reaction mixture. Other promutagens were dissolved in water. After the incubation at 37° for 5 min, 10 μM of vitamin K_3 (9 nmole/ml) was added to the reaction mixture to stop the reaction. The mixture was centrifuged at 1500 *g* for 10 min, and then the resulting pellet was suspended in 0.1 M potassium phosphate buffer (pH 7.4). The bacteria cells were washed again by recentrifugation at 2000 *g* for 30 min, and resuspension in 0.1 M potassium phosphate buffer (pH 7.4). The suspension was mixed with molten soft agar containing a limited amount of L-histidine and biotin, and then the mixture was poured onto a Petri dish containing 25 ml of minimal glucose agar. The number of spontaneous revertants was subtracted from the experimental values. Other details were the same as described elsewhere [6].

Other methods. P-450c and P-450d were purified from liver microsomes of PCB-treated rats [6]. Protein concentration was estimated by the method of Lowry *et al.* [16]. The content of cytochrome P-450 was measured according to the method of Omura and Sato [17]. NADPH-cytochrome P-450 reductase was purified from liver microsomes [18] of phenobarbital-treated rats to a specific activity of 27.5 unit per mg of protein. The reductase activity was determined by the method of Phillips and Langdon [19] using cytochrome *c* as an electron acceptor. One unit of reductase was defined as the amount of reductase catalyzing the reduction of one μmol of cytochrome *c* per min. SDS-PAGE and Western blot-peroxidase antiperoxidase (PAP) staining were carried out according to the methods of Laemmli [20] and Guengerich *et al.* [21], respectively. Antibodies against P-450d was raised in rabbits as described [22]. The *N*-terminal amino acid sequence of P-450-D3 was analyzed by Edman degradation using automatic gas phase sequencer (Applied Biosystems, Model 477A) after the P-450-D3 preparation was dialyzed thoroughly against water and lyophilized.

RESULTS

The elution profile of cytochrome P-450 from an ω-aminooctyl Sepharose 4B column is shown in Fig. 1. Cytochrome P-450 immunochemically crossreactive with anti-P-450d antibodies was eluted into the fractions shown by oblique lines. Figure 2 shows the elution profile of cytochrome P-450 from a preparative DEAE-5PW column. Each fraction was subjected to immunoblotting with anti-P-450d antibodies to examine the crossreactive protein before fractions were combined. Cytochrome P-450 immunochemically reactive with anti-P-450d antibodies was eluted into the fractions shown by oblique lines. Summary of a typical purification of cytochrome P-450, designated as P-450-D3, from liver microsomes of PCB-treated female Beagle dogs, is shown in Table 1. Specific content of the purified P-450-D3 was 13.3 nmole/mg of protein, and the

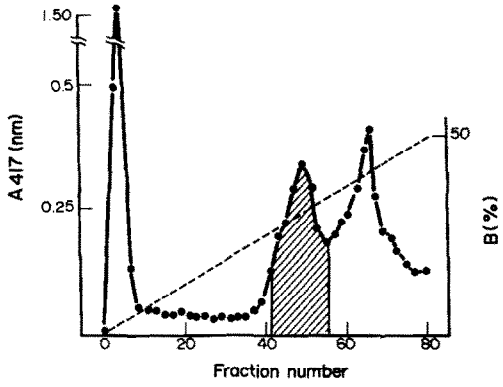

Fig. 1. Elution profile of cytochrome P-450 from aminooctyl Sepharose 4B column. After the column was washed with 10 mM K–phosphate buffer (pH 7.4) containing 20% glycerol, 1 mM DTT, 0.1 mM EDTA, 0.5% sodium cholate and 0.2% Emulgen 911 (Buffer A), cytochrome P-450 was eluted by washing the column with linear gradients of the concentrations of potassium phosphate (10 mM to 200 mM) and Emulgen 911 (0.2% to 0.5%). Per cent of B, shown by the dotted line, represents the percentage of Buffer B which contains 200 mM potassium phosphate buffer (pH 7.4), 20% glycerol, 1 mM DTT, 0.1 mM EDTA, 0.5% sodium cholate and 0.5% Emulgen 911. The eluate was collected to 15 ml fractions and monitored for cytochrome P-450 with absorbance at 417 nm.

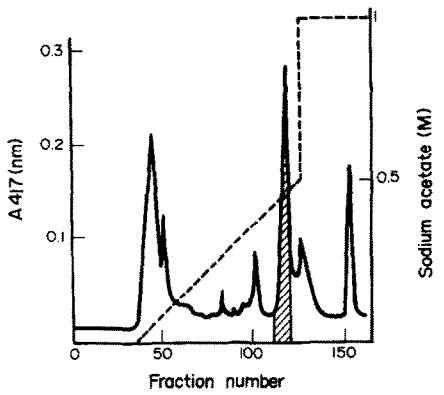

Fig. 2. HPLC elution profile of cytochrome P-450 using a preparative DEAE-5PW column. After the column was washed with 20 mM Tris–acetate buffer (pH 7.5) containing 20% glycerol and 0.4% Emulgen 911, cytochrome P-450 was eluted by washing the column with a linear gradient of the concentration of sodium acetate. The dotted line represents the concentration of sodium acetate, and the eluate was collected to 1 ml fractions, and monitored for cytochrome P-450 with absorbance at 417 nm.

recovery of the cytochrome was approximately 0.9% of the total cytochrome P-450 in liver microsomes.

As shown in Fig. 3, the final preparation of P-450-D3 gave a single protein band on SDS-PAGE. When compared to standard proteins (bovine serum albumin, ovalbumin and carbonic anhydrorase), an apparent molecular weight of P-450-D3 was estimated to be 54,000.

As can be seen in Fig. 4, the carbon monoxide bound reduced form of P-450-D3 showed a peak at 448 nm. The oxidized form of P-450-D3 showed a peak at 416 nm, indicating that P-450-D3 is mostly in a low spin state.

The drug oxidation activities of P-450-D3 were compared to those of P-450-D2, P-450c and P-450d. As can be seen in Table 2, the *N*-demethylase activities of P-450-D3 for benzphetamine and aminopyrine were higher than those of P-450c and P-450d, and were comparable with those of P-450-D2. The rate of aniline hydroxylation catalyzed by P-450-D3 was similar to that seen with P-450-D2 or P-450c but was much lower than that with P-450d. The catalytic activities of P-450-D3 for 7-ethoxycoumarin *O*-deethylation and benzo(*a*)pyrene hydroxylation, which are catalyzed efficiently by a low spin form of cytochrome P-448 in rats, were considerably lower than those of P-450c. Furthermore, the catalytic activity of P-450-D3 for *p*-propoxyaniline *O*-depropoxylation was similar to that of P-450d but higher than that of P-450c. These results suggest the possibility that the catalytic activities of P-450-D3 for drug oxidations are similar to those of P-450d rather than P-450c except that the rate of aniline *p*-hydroxylation by P-450-D3 was lower than that by P-450d.

Since P-450c and P-450d have been demonstrated to catalyze mutagenic activation of promutagens such as Trp-P-2, Glu-P-1, aflatoxin B_1 and benzo(*a*)pyrene, the activity of P-450-D3 for metabolic activation of these promutagens was investigated in a reconstituted system. As shown in Table 3, P-450-D3 catalyzed mutagenic activation of promutagens such as Glu-P-1, aflatoxin B_1 and benzo(*a*)pyrene as did P-450-D2. P-450-D3 activated all of these promutagens tested at rates slower than did P-450c and P-450d, while the rates may be still toxicologically significant.

Table 1. Summary of purification of P-450-D3 from liver microsomes of PCB-treated female Beagle dogs

Purification step	Total protein (mg)	Cytochrome P-450		
		Total content (nmole)	Specific content (nmole/mg)	Recovery (%)
Microsomes	1,500	2,200	1.45	100
Solubilized supernatant	960	1,800	1.88	82.2
Aminooctyl Sepharose 4B eluate	32.9	280	8.50	12.7
DEAE-5PW eluate (P-450-D3)	1.5	20	13.3	0.9

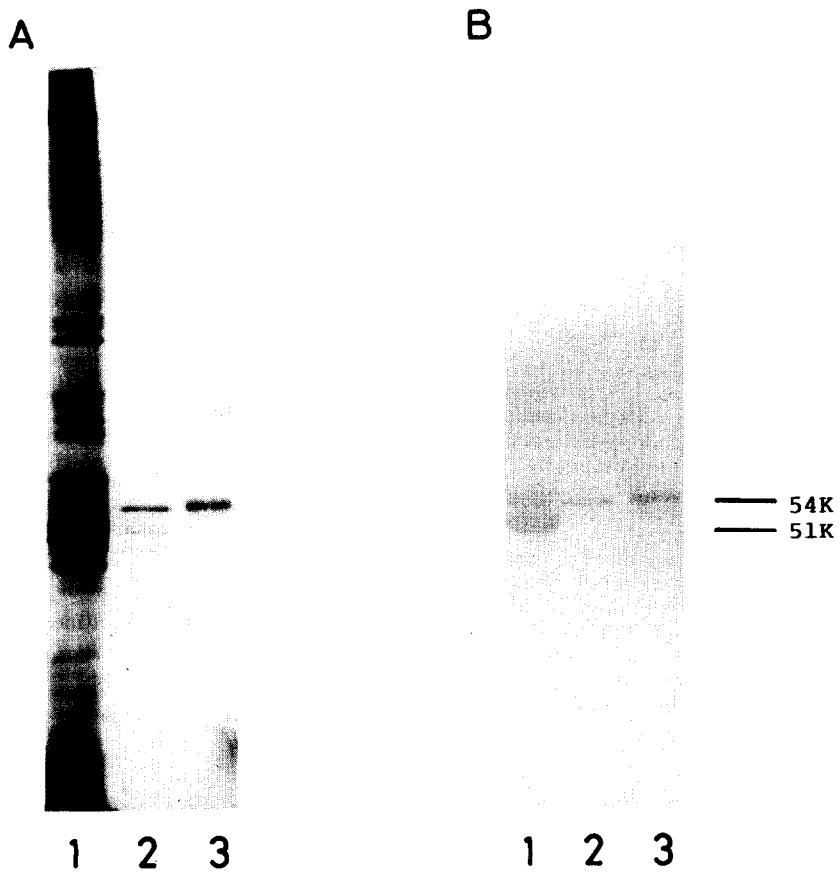

Fig. 3. SDS-PAGE and Western Blot analysis of P-450-D3 with anti-PCB P-448-H antibodies. SDS-PAGE (A) and Western blot analysis (B) were performed as described in Materials and Methods. Lanes 1 to 3 contain solubilized liver microsomes, a pooled fraction eluted from an ω-aminooctyl Sepharose 4B column and purified P-450-D3, respectively.

It is known that the amino acid sequences of *N*-terminal region varies among forms of cytochrome P-450. Thus, the sequences of this peptide region have been determined to know the homology with other forms of cytochrome P-450. As can be seen in Fig. 5, the first 16 amino terminal sequence of P-450-D3 was found to be highly homologous to that of a high spin form of cytochrome P-450, designated as P-450-D2, which has been previously purified from PCB-treated female Beagle dog [8]. As in the case of P-450-D2, the amino terminal portion of P-450-D3 was also found to be similar to those of P-450d, human P_3-450 and P_3-450 when four amino acid deletions were tentatively inserted between fifth and sixth amino acids from the *N*-terminal amino acid. The amino terminal sequence of P-450-D3 was considerably different from that of P-450c.

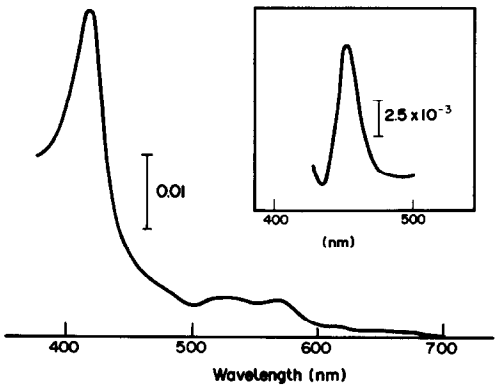

Fig. 4. Spectral properties of P-450-D3 purified from PCB-treated female Beagle dogs. Absolute spectrum of oxidized form was measured in the presence of 20 mM potassium phosphate buffer (pH 7.4) containing 20% glycerol and 0.2% Emulgen 911. Spectrum framed in the figure represents the carbon monoxide bound reduced minus reduced difference spectrum of purified P-450-D3.

DISCUSSION

PCB-inducible families of cytochrome P-450 in liver microsomes contain at least two proteins, P-450c and P-450d, that are immunochemically related. These forms of cytochrome P-450 are responsible for the metabolic activation of a number of mutagenic or carcinogenic compounds [6]. In this report, we purified a low spin form of cytochrome P-450, designated as P-450-D3, to an electrophoretic homogeneity as judged by SDS-PAGE from liver

Table 2. Drug metabolizing enzyme activities of P-450-D3 in a reconstituted system

Substrate	Concentration (mM)	Cytochrome P-450			
		P-450-D3	P-450-D2	P-450c	P-450d
		(nmole/min/nmole P-450)			
Benzphetamine	5	48.6	39.2	19.6	23.1
Aminopyrine	5	45.2	38.3	20.4	30.1
Aniline	5	0.58	0.35	0.70	5.51
p-Propoxyaniline	5	6.30	5.16	1.49	4.81
7-Ethoxycoumarin	0.5	4.36	4.38	123	1.60
Benzo(*a*)pyrene	0.04	0.83	0.18	6.45	0.042

Each value represents the mean of duplicate determinations. P-450c and P-450d were purified from liver microsomes of PCB-treated rats.

Table 3. Mutagenic activation of promutagens by P-450-D3 in a reconstituted system

Promutagens	Concentration (μM)	Tester strain	Cytochrome P-450			
			P-450-D3	P-450-D2	P-450c	P-450d
			(revertants $\times 10^{-3}$/nmole P-450)			
Trp-P-2	500	TA98	25		116	200
Glu-P-1	500	TA98	37	83	56	110
Aflatoxin B_1	2	TA100	0.6	4.6	3.7	8.8
Benzo(*a*)pyrene	40	TA100	1.3	1.0	4.7	1.7

Each value represents the mean of duplicate determinations. The number of spontaneous revertants in the absence of NADPH-generating system ranged from 0.03 to 0.04. P-450c and P-450d were purified from liver microsomes of PCB-pretreated rats as described in Materials and Methods.

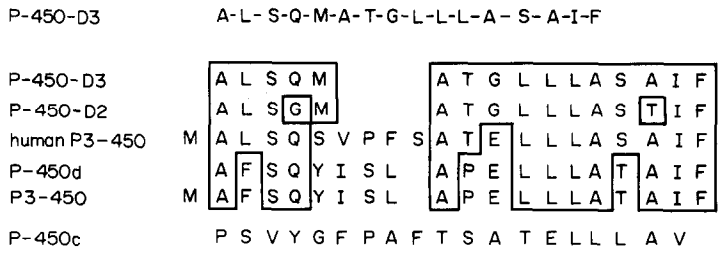

Fig. 5. N-Terminal amino acid sequence of P-450-D3 purified from liver microsomes of PCB-treated female Beagle dogs. 0.3 pmole of purified P-450-D3 was subjected to automatic liquid phase sequencer. Amino acid sequences obtained from the following sources in parentheses: human P_3-450 [23], P-450c and P-450d [24], P_3-450 [25]. Frames are those residues identical with the corresponding residues of P-450-D3.

microsomes of PCB-treated female Beagle dogs. The purified P-450-D3 showed catalytic activities for mutagenic activation of promutagens indicating that this form may be, at least in part, responsible for the metabolic activation in liver microsomes of PCB-treated female Beagle dogs. The purified P-450-D3 was in a low spin state and was distinguishable from P-450d in their molecular weights, whereas *N*-terminal 20 amino acid residues of P-450-D3 was highly similar to those of P-450d rather that those of P-450c when four amino acid residues were tentatively inserted between fifth and sixth from the *N*-terminal amino acid of P-450-D3. In these reports, we propose that P-450-D3 is a novel form of cytochrome P-450 distinct from those which have been previously purified from liver microsomes.

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REFERENCES

1. Nebert DW and Negishi M, Multiple forms of cytochrome P-450 and the importance of molecular biology and evolution. *Biochem Pharmacol* **31**: 2311–2317, 1982.
2. Lu AYH and West SB, Multiplicity of mammalian cytochrome P-450. *Pharmacol Rev* **31**: 277–295, 1980.
3. Amacher DE and Smith DJ, Differences in the constitutive form of hepatic cytochrome P-450 in male and

female adult Beagle dogs. *J Chromatogr* **419**: 61–73, 1987.
4. Duignan DB, Gleensipes I, Leonard TB and Harpert JR, Purification and characterization of the dog hepatic cytochrome P-450 isozyme responsible for the metabolism of 2,2′,4,4′,5,5′-hexachlorobiphenyl. *Arch Biochem Biophys* **255**: 290–303, 1987.
5. Ryan DE, Thomas PE, Reik LM and Levin W, Purification, characterization and regulation of five rat hepatic microsomal cytochrome P-450 isozymes. *Xenobiotica* **12**: 727–744, 1982.
6. Kamataki T, Maeda K, Yamazoe Y, Matsuda N, Ishii K and Kato R, A high-spin form of cytochrome P-450 highly purified from polychlorinated biphenyl-treated rats. Catalytic characterization and immunochemical quantitation in liver microsomes. *Mol Pharmacol* **24**: 146–155, 1983.
7. Nebert DW, Adesnik M, Coon MJ, Estabrook RW, Gonzalez FJ, Guengerich FP, Gunsalus IC, Johnson EF, Kemper B, Levin W, Phillips IR, Sato R and Waterman MR, The P450 gene superfamily: Recommended nomenclature. *DNA* **6**: 1–11, 1987.
8. Motoya M, Komori M, Miura T and Kamataki T, Purification and characterization of a high spin form of cytochrome P-450 from liver microsomes of PCB-treated Beagle dogs. *Seikagaku* **59**: 871, 1987.
9. Kamataki T and Kitagawa H, Effects of lyophilization and storage of rat liver microsomes on activity of aniline hydroxylase, contents of cytochrome b_5 and cytochrome P-450 and aniline-induced P-450 difference spectrum. *Japan J Pharmacol* **24**: 195–203, 1974.
10. Imai Y and Sato R, A gel-electrophoretically homogeneous preparation of cytochrome P-450 from liver microsomes of phenobarbital-pretreated rabbits. *Biochem Biophys Res Commun* **60**: 8–14, 1974.
11. Funae Y and Imaoka S, Simultaneous purification of multiple forms of rat liver microsomal cytochrome P-450 by high-performance liquid chromatography. *Biochim Biophys Acta* **842**: 119–132, 1985.
12. Nash T, The colorimetric estimation of formaldehyde by means of the Hantzsh reaction. *Biochem J* **55**: 416–421, 1953.
13. Aitio A, A simple and sensitive assay of 7-ethoxycoumarin deethylation. *Anal Biochem* **85**: 488–491, 1978.
14. Nebert DW and Gelboin HV, Substrate inducible microsomal arylhydroxylase in mammalian cell culture. *J Biol Chem* **243**: 6242–6249, 1968.
15. Imai Y, Ito A and Sato R, Evidence for biochemically different types of vesicles in hepatic microsomal fraction. *J Biochem* **60**: 417–428, 1966.
16. Lowry OH, Rosebrough HJ, Farr AL and Randall RJ, Protein measurement with the Folin phenol reagent, *J Biol Chem* **193**: 265–275, 1951.
17. Omura T and Sato R, The carbon monooxide-binding pigment of liver microsomes I. Evidence for its hemoprotein nature. *J Biol Chem* **239**: 2370–2378, 1964.
18. Yasukochi Y and Masters BSS, Some properties of a detergent-solubilized NADPH-cytochrome *c* (cytochrome P-450) reductase purified by biospecific affinity chromatography. *J Biol Chem* **251**: 5337–5344, 1976.
19. Phillips AH and Langdon RG, Hepatic triphosphopyridine nucleotide-cytochrome *c* reductase: Isolation, characterization and kinetic studies. *J Biol Chem* **237**: 2652–2660, 1962.
20. Laemmli UK, Cleavage of structure proteins during the assembly of the head of bacteriophage T4. *Nature (Lond)* **227**: 680–685, 1970.
21. Guengerich FP, Wang P and Davidson NK, Estimation of isozymes of microsomal cytochrome P-450 in rats, rabbits and humans using immunochemical staining coupled with sodium dodecylsulfate-polyacrylamide gel electrophoresis. *Biochemistry* **21**: 1698–1706, 1982.
22. Kamataki T, Belcher DH and Neal RA, Studies of the metabolism of diethyl *p*-nitrophenyl phosphorothionate (parathion) and benzphetamine using an apparently homogeneous preparation of rat liver cytochrome P-450: Effect of a cytochrome P-450 antibody preparation. *Mol Pharmacol* **12**: 921–932, 1976.
23. Jaiswall K, Nebert DW and Gonzalez FT, Human P_3-450: cDNA and complete amino acid sequence. *Nucleic Acids Res* **14**: 6773–6774, 1986.
24. Haniu M, Ryan S, Ida S, Lieber CS, Levin W and Shively JE, NH_2-terminal sequence analyses of four rat hepatic microsomal cytochrome P-450. *Arch Biochem Biophys* **235**: 304–311, 1984.
25. Gonzalez FJ, Mackenzie PI, Kimura S and Nebert DW, Isolation and characterization of full-length mouse cDNA and genomic clones of 3-methylcholanthrene-inducible cytochrome P-450 and P_3-450. *Gene* **29**: 281–292, 1984.