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ω-1 and ω-2 Hydroxylation of Prostaglandins by Rabbit Hepatic Microsomal Cytochrome *P*-450 Isozyme 6[1]

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Incubation of prostaglandin E_1 (PGE_1) with liver microsomes from control rabbits and from rabbits treated with ethanol or imidazole yielded 18-, 19-, and 20-hydroxy metabolites, representing hydroxylation at ω-2, ω-1, and ω carbons, respectively. The current investigation demonstrates that rabbit liver *P*-450 isozyme 6 effectively catalyzes the ω-1 and ω-2 hydroxylation of PGE_1 and PGE_2. Additionally, a small amount of product with chromatographic characteristics of the corresponding 20-hydroxy metabolite has been detected. The incorporation of cytochrome b_5 into the reconstituted system did not enhance the rate of PGE_1 hydroxylation and had no effect on the ratio of products formed. The K_m value for the ω-1 and ω-2 hydroxylation of PGE_1 with *P*-450 isozyme 6 from imidazole-treated rabbits was approximately 140 μM; the $V_{max's}$ (nmol product min^{-1} nmol *P*-450^{-1}) were 2.1 and 1.1 for the ω-1 and ω-2 hydroxylations, respectively. These rates represent the highest activities by hepatic *P*-450 isozymes for hydroxylation of PGs, and suggest that isozyme 6 is responsible for the ω-2 hydroxylation of PGEs observed in rabbit liver microsomes.

Evidence has accumulated that the urinary prostaglandins (PGs)[5] oxidized at the terminal (ω) and the penultimate (ω-1) carbons are products of monooxygenase-mediated hydroxylation of primary PGs (3-6). It was observed that rabbit and other mammalian liver microsomal cytochrome *P*-450 monooxygenases catalyze the hydroxylation of prostaglandins (PGs) at the ω and ω-1 carbons (6-11). Subsequent studies with purified cytochrome *P*-450s from rabbit liver demonstrated a high degree of regioselectivity of hydroxylation by certain isozymes (12). Whereas isozyme 4 catalyzed solely the ω-1 hydroxylation, isozyme 2, requiring cytochrome b_5, catalyzed PG hydroxylation primarily at ω-1 and to a small extent at the ω position; isozymes 3a, 3b, and 3c had only little catalytic activity toward PGs (12). Additionally, form 7 exhibited exclusive ω hydroxylation (30).

The current investigation demonstrates that, in addition to hydroxylation at the ω- and ω-1 positions, rabbit liver microsomes

[1] Preliminary reports of parts of this investigation have been presented at conferences (1, 2).

[2] In partial fulfillment of PhD requirements at the Chemistry Department of Clark University, Worcester, Mass.

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[5] Abbreviations used: PGs, prostaglandins; PGE_1 and PGE_2, prostaglandins E_1 and E_2; ω, C_{20}; ω-1, C_{19}; ω-2, C_{18}; dilauroyl-GPC, dilauroylglyceryl-3-phosphorylcholine; PI, 1-phenylimidazole; IPI, 1-phenyl-(2-isopropyl)-imidazole; BUDMS, *t*-butyldimethylsilyl; TMS, trimethylsilyl; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TCDD, tetrachlorodibenzo-*p*-dioxin.

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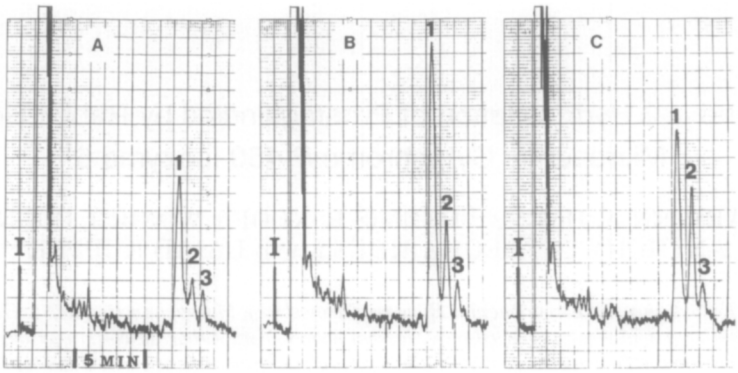


FIG. 1. HPLC profile of 10% of the extract from incubations of PGE_1 (0.5 mM) with liver microsomes from rabbits; PGE_1 and metabolites were converted to PGB derivatives to facilitate detection at 280 nm (10). A Whatman C_8 (5 μm) column was used. The mobile phase was 25% CH_3CN/75% H_2O (containing 0.01% acetic acid) at 2 ml/min. Monitoring was at 280 nm (0.005 AUFS). (A) Control rabbits; (B) ethanol-treated rabbits; and (C) imidazole-treated rabbits. Incubations, as described under Experimental Procedures, were conducted for 30 min at 37°C and were terminated by addition of ethanol; the subsequent workup was as previously described (10). Notations: I, injection point; peak 1, 19-OH-PGB_1; peak 2, 20-OH-PGB_1; peak 3, 18-OH-PGB_1.

also catalyze the hydroxylation of PGs at the ω-2 position.[6] Moreover, a reconstituted system composed of *P*-450 isozyme 6, NADPH-*P*-450 reductase, and dilauroyl-GPC effectively catalyzes the hydroxylation of PGs at both ω-2 and ω-1 sites, suggesting that isozyme 6 is responsible for the ω-2 hydroxylation observed in liver microsomes. Additionally, the above findings suggest that the various sites of hydroxylation of prostaglandins could be used to probe for the presence and activity of individual *P*-450 isozymes in liver microsomes.

EXPERIMENTAL PROCEDURES

Liver microsomes were prepared from rabbits treated with ethanol, isosafrole, or imidazole (21). Cytochrome *P*-450 isozyme 6 was isolated from rabbit liver microsomes and purified to homogeneity by SDS-PAGE as previously described (22). NADPH-cytochrome *P*-450 reductase (23) and cytochrome b_5 (24) were isolated from rabbit liver microsomes. Incubations of [^{3}H] PGE_1 (0.5 mM, 0.05 μCi) and [^{3}H] PGE_2 (0.5 mM, 0.05 μCi) were carried out with liver microsomes (0.6 mg protein) and an NADPH regenerating system (10), or with a reconstituted system composed of *P*-450 isozyme 6 (usually 0.25 μM), reductase (0.25 or 0.38 μM), and dilauroyl-GPC (30 μg/ml) in 0.1 M potassium phosphate (pH 7.4); the final volume was 0.9 ml. After a 3-min equilibration period at 30°C, the reaction was initiated with 0.1 ml of NADPH solution (2 μmol) and was conducted in a Dubnoff shaker at 30°C (for incubation time, see tables). The reaction was terminated by addition of 10 ml of ethanol followed by sedimentation of protein in a clinical centrifuge. The supernate was evaporated to dryness under nitrogen. Isolation of metabolites as PGB derivatives was obtained by HPLC using a C_8 reversed-phase column (10, 19). For identification, the eluted metabolites were converted to methyl esters with diazomethane and purified on HPLC. The methyl ester derivatives were converted to *t*-butyldimethylsilyl (BuDMS) or to trimethylsilyl (TMS) ethers as previously described (10). The BuDMS derivatives were further purified by HPLC. The purified BuDMS ether and the freshly prepared TMS ether methyl ester PGB derivatives were subjected to GC/MS (10, 19).

RESULTS AND DISCUSSION

Incubations of PGE_1 with liver microsomes from control, ethanol-treated, and imidazole-treated rabbits yielded in each

[6] In previous investigations, using a "fatty acid" reverse-phase column, the ω-2-hydroxy products cochromatographed with the corresponding ω-hydroxy products, and hence remained undetected.

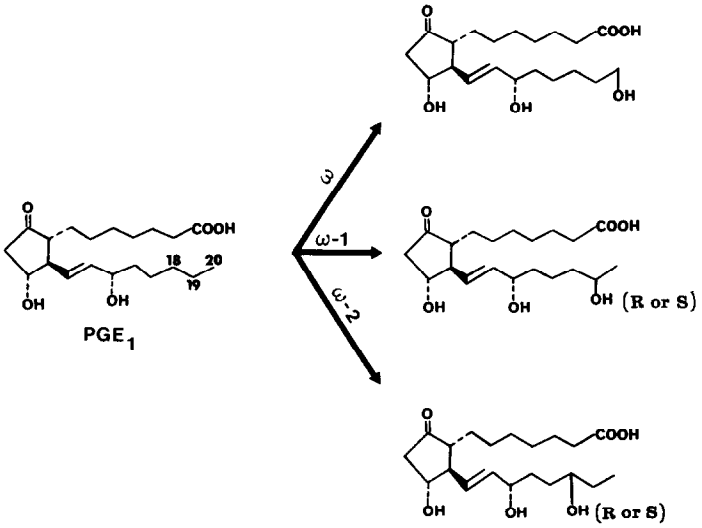


FIG. 2. Structures of PGE_1 metabolites.

case three chromatographically separable metabolites (Fig. 1) which correspond in retention time to 18-, 19-, and 20-hydroxy-PG derivatives; thus representing ω-2, ω-1, and ω hydroxylation of PGE_1, respectively (Fig. 2). The identity of the 19- and 20-hydroxy metabolites was established by comparison with authentic compounds on reversed-phase HPLC (10). The 18-hydroxy product was identified by comparison of chromatographic retention time with a recently characterized 18-hydroxy product derived from incubations of PGE_1 with rat liver microsomes (19). As can be seen qualitatively in Fig. 1, treatment of rabbits with ethanol or imidazole does not have a significant effect on the formation of 18-OH-PGE_1. The activity of isozymes 2, 3a, 3b, 3c, and 4 toward PGE_1 has been reported (12), and recently form 7 was shown to ω-hydroxylate PGE_1 and PGE_2 (30); none of these isozymes exhibited any ω-2 hydroxylase activity. Recent experiments have demonstrated that the major 3-methylcholanthrene-inducible form of *P*-450 from rats [*P*-450c or allozymes from different rat strains (14, 25) is probably responsible for the ω-2 hydroxylating activity in that species (19)]. As judged by sequence homology, rabbit isozyme 4 is structurally related to rat *P*-450d, while rabbit isozyme 6 is related to rat *P*-450c (26, 29).[7] Similar conclusions were reached when the cross-

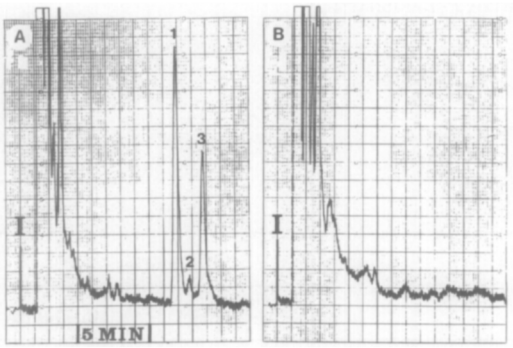
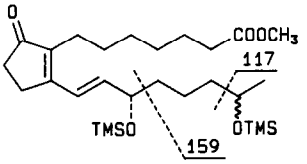


FIG. 3. HPLC profile of metabolites of PGE_1 (as PGB derivatives) from incubation with isozyme 6 in a reconstituted system (see Experimental Procedures). The complete system consisted of *P*-450 isozyme 6 (0.25 μM) isolated from ethanol-treated rabbits, reductase (0.375 μM), and dilauroyl-GPC (30 μg). The reaction was initiated with NADPH (2 mM) for 10 min at 30°C. HPLC was conducted as in Fig. 1. (A) Complete system; and (B) complete system minus NADPH-*P*-450 reductase. Notations: I, injection point; peak 1, 19-OH-PGB_1; peak 2, 20-OH-PGB_1; peak 3, 18-OH-PGB_1.

[7] V. S. Fujita, D. R. Koop, and M. J. Coon, unpublished results.

TABLE IA

ION ASSIGNMENT AND INTENSITIES IN THE MASS SPECTRUM OF THE TRIMETHYLSILYL ETHER METHYL ESTER OF THE AUTHENTIC 19-OH-PGB_1 DERIVATIVE AND OF THE HPLC-PURIFIED MOST POLAR METABOLITE FROM PGE_1 INCUBATION WITH *P*-450 ISOZYME 6

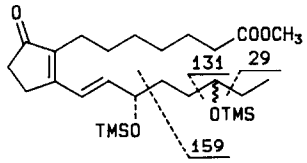


Ion assignment (m/z)			Intensities (%)[a]	
			Authentic[b]	Metabolite
510		M+	94	62
420	M^+-90 =	M^+-[TMSOH]	15	10
392	M^+-[117 + 1] =	M^+-[$CH_3CHOTMS$ + 1]	30	15
351	M^+-159 =	M^+-[$CH_3CH(OTMS)CH_2CH_2CH_2$]	76	55
323	M^+-187	Unidentified	100	100
117		[$CH_3CHOTMS$]	70	51

[a] Estimated from the computer-generated mass fragmentation pattern.
[b] Authentic 19-OH-PGB_1 was prepared from human semen (20).

TABLE 1B

ION ASSIGNMENT AND INTENSITIES IN THE MASS SPECTRUM OF THE TRIMETHYLSILYL ETHER METHYL ESTER OF THE AUTHENTIC 18-OH-PGB_1 DERIVATIVE AND OF THE HPLC-PURIFIED LEAST POLAR METABOLITE FROM PGE_1 INCUBATION WITH *P*-450 ISOZYME 6



Ion assignment (m/z)			Intensities (%)[a]	
			Authentic[b]	Metabolite
510		M^+	81	31
420	M^+-90 =	M^+-[TMSOH]	30	29
391	M^+-[90 + 29] =	M^+-[TMSOH + CH_3CH_2]	27	21
364	M^+-[131 + 15] =	M^+-[$CH_3CH_2CHOTMS$ + CH_3]	49	50
351	M^+-159 =	M^+-[$CH_3CH_2CH(OTMS)CH_2CH_2$]	50	21
323	M^+-187	unidentified	100	62
131		[$CH_3CH_2CHOTMS$]	45	34

[a] Estimated from the computer-generated mass fragmentation pattern.
[b] Authentic 18-OH-PGB_1 was obtained from incubations of PGE_1 with liver microsomes from MC-treated rats (19).

reactivity of monoclonal antibodies to *P*-450c and *P*-450d was examined with rabbit hepatic microsomes and purified isozymes 4 and 6 (27).

Incubation of PGE_1 with the reconstituted system *P*-450 isozyme 6 from rabbit liver yielded primarily 18- and 19-hydroxylated PGE_1 metabolites and a minor product with chromatographic characteristics of the 20-hydroxy derivative (Fig. 3A). There was no hydroxylating activity in the absence of NADPH-*P*-450 reductase (Fig. 3B). Identification of the above products as 18- and 19-hydroxy products was achieved by coinjection with authentic compounds on HPLC and by GC/MS of the corresponding BuDMS [not shown, see Ref. (19)] and TMS derivatives (Tables IA and B). Similar results were obtained when PGE_2 was incubated with isozyme 6 [for mass fragments assignment see Ref. (19)]. A molar ratio of 1.5-2.0 NADPH-*P*-450 reductase to *P*-450 isozyme 6 resulted in optimal rates of ω-1 and ω-2 hydroxylation of PGE_1 (Fig. 4). Linearity of product formation was observed up to 10 min with slight deviation from linearity for an additional 10 min, and the rates of ω-1 and ω-2 hydroxylations were linear with concentration of up to 0.5 μM isozyme 6 (Fig. 5). PGE_2 is metabolized by isozyme 6 at a much lower rate than PGE_1; nevertheless

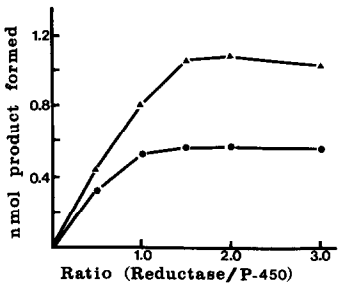


FIG. 4. Determination of the optimal ratio of NADPH-*P*-450 reductase to *P*-450 isozyme 6 for PGE_1 hydroxylation. Isozyme 6 (0.25 μM) was from ethanol-treated rabbits; the reductase concentration ranged from 0.125 to 0.750 μM. The rest of the constituents and incubation conditions were as described under Experimental Procedures, except that the incubation was for 5 min. ●, 18-OH; ▲, 19-OH.

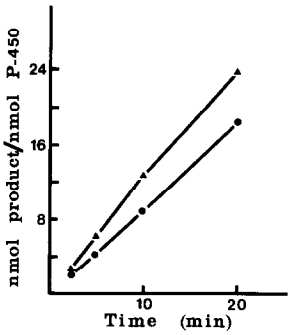


FIG. 5. Time course of PGE_1 hydroxylation by *P*-450 isozyme 6. The reconstituted system consisted of isozyme 6 (0.25 μM) from imidazole-treated rabbits, reductase (0.375 μM), and the rest of the constituents and incubation conditions as in Fig. 3. ●, 18-OH; ▲, 19-OH.

the ratio of the two metabolites is equivalent to that observed with PGE_1 (Table II). Since cytochrome b_5 was obligatory for catalysis by isozyme 2 and stimulated the rate and extent of hydroxylation of PGs by isozyme 4 (12), the effect of b_5 on catalysis by isozyme 6 was examined. The addition of cytochrome b_5 to incubations containing isozyme 6 from ethanol- or imidazole-treated rabbits diminished PGE_1 hydroxylation (Table III). Earlier studies showed that the mode and sequence of b_5 addition to the reconstituted system play a role in whether inhibition of monooxygenase by b_5 is manifested (31). In fact, we observed that the addition of a dilute solution of b_5 after dilauroyl-GPC did not inhibit PGE_1 hydroxylation (not shown).

A comparison of activity of isozyme 6 isolated from rabbits treated with ethanol, isosafrole, and imidazole demonstrated that imidazole treatment yielded the most active form of isozyme 6 (Table II), as well as the most highly purified preparation. The results of experiments (not shown) suggest that it is unlikely that a heat-stable, nonvolatile inhibitor is responsible for the much lower activity of isozyme 6 from ethanol-treated than from imidazole-treated rabbits. The addition of heat-inactivated isozyme 6 from ethanol-treated rabbits to isozyme 6 from imidazole-treated rabbits did not affect the activity

TABLE II

HYDROXYLATION OF PGE_1 and PGE_2 BY *P*-450 ISOZYME 6 FROM LIVERS OF RABBITS TREATED WITH ETHANOL, ISOSAFROLE, OR IMIDAZOLE

Experiment	Source of *P*-450 (treatment)	Substrate	Incubation (min)	PGE metabolites[b] nmol min^{-1} nmol *P*-450^{-1} 19-OH	18-OH	Ratio 19-OH/18-OH
1	Ethanol	PGE_1	5	0.75	0.44	1.70
		PGE_1	10	0.74	0.41	1.80
2	Isosafrole[a]	PGE_1	5	0.84	0.49	1.71
		PGE_1	10	0.65	0.44	1.48
3	Imidazole	PGE_1	5	1.27	0.86	1.47
		PGE_1	10	1.28	0.89	1.44
4	Imidazole	PGE_2	5	0.34	0.22	1.54
		PGE_2	10	0.37	0.22	1.68

Note. Each incubation contained PGE_1 or PGE_2 (0.5 mM) and *P*-450 isozyme 6 (0.25 μM), dilauroyl-GPC (30 μg), NADPH (2 mM), and *P*-450 reductase (0.25 μM, Exp. 2; 0.38 μM, Exp. 1, 3, 4). Isozymes 6 from ethanol, isosafrole, and imidazole treatments had specific contents of 13.3, 9.5, and 16.2 nmol/mg protein.

[a] Experiment 2 exhibited lack of linearity with time (2.5–20 min); for comparison, only the 5- and 10-min incubations are presented.

[b] 20-Hydroxy derivatives were not quantitated because the levels were too low for accurate determination.

of the latter. Also the addition of heat-inactivated isozyme 6 from imidazole-treated to isozyme 6 from ethanol-treated rabbits did not affect the enzymatic activity, indicating the absence of an activator in the preparation from imidazole-treated rabbits. Finally, a "mixing" experiment of equal amounts of active preparations of isozyme 6 from ethanol-treated and imidazole-treated rabbits demonstrated addi-

TABLE III

EFFECT OF CYTOCHROME b_5 ON PGE_1 HYDROXYLATION BY *P*-450 ISOZYME 6

Experiment	Additions	Incubation time (min)	nmol min^{-1} nmol P-450^{-1} 19-OH	18-OH
1	—	5	0.75	0.44
	b_5	5	0.59 (0.79)	0.34 (0.77)
	—	10	0.74	0.41
	b_5	10	0.58 (0.78)	0.36 (0.88)
2	—	10	1.58	0.82
	b_5	10	1.13 (0.72)	0.58 (0.71)

Note. Each incubation contained PGE_1 (0.5 mM). Components were added in the following order: *P*-450 isozyme 6 (0.25 μM), NADPH-*P*-450 reductase (0.38 μM), and dilauroyl-GPC (30 μg). Reaction was initiated with NADPH (2 mM). Cytochrome b_5 (0.25 μM) was added prior to dilauroyl-GPC. Experiment 1 contained isozyme 6 (13.3 nmol/mg protein) isolated from ethanol-treated rabbits and Experiment 2 contained isozyme 6 (16.2 nmol/mg protein) isolated from imidazole-treated rabbits. Values in parentheses represent ratios of products from incubations containing b_5 to those lacking b_5.

TABLE IV

EFFECT OF MONOOXYGENASE INHIBITORS ON THE RATE OF HYDROXYLATION OF PGE_1 BY *P*-450 ISOZYME 6

Inhibitor[a] (μM)	nmol Product min^{-1} nmol *P*-450^{-1} 19-OH	18-OH	Ratio 19-OH/18-OH
—	1.63	0.91	1.79
PI (10)	0.88 (54)[b]	0.48 (53)	1.83
IPI (10)	0.30 (18)	0.18 (20)	1.67
SKF525A (100)	0.22 (13)	0.12 (13)	1.83
Ethanol (172 mM)	0.64 (39)	0.33 (36)	1.94
Miconazole (10)	0	0	

Note. Each incubation contained 0.23 mM PGE_1. Isozyme 6 had a specific content of 16.2 nmol/mg protein and was isolated from imidazole-treated rabbits. Other constituents were as in Table III. Values represent the mean of duplicates, except in the control (no inhibitor) and the ethanol, which were means of triplicates. 20-OH formation was detected (0.04-0.12 nmol min^{-1} nmol *P*-450^{-1}).

[a] The inhibitors were added in water except for Miconazole, which was added in 10 μl ethanol.

[b] Values in parentheses are percentages of control.

tive effects (not shown). Additionally, the observation that the addition of catalase had no effect on the rates of ω-2 and ω-1 hydroxylation of PGE_1 by isozyme 6 preparations from ethanol-treated or imidazole-treated rabbits (not shown) indicates that H_2O_2 formation is not responsible for the difference in activities of the two preparations of isozyme 6.

Incubation of PGE_1 with isozyme 6, from imidazole-treated rabbits, in the presence of various monooxygenase inhibitors did not demonstrate preferential inhibition of hydroxylation at the various sites (Table IV). A K_m value of approximately 140 μM was observed for the 19- and 18-hydroxylations of PGE_1 with isozyme 6 from imidazole-treated rabbits; the $V_{max's}$ were 2.1 and 1.1 nmol min^{-1} nmol *P*-450^{-1} for 19-OH and 18-OH, respectively (Fig. 6). The variation in the ratio of 19- to 18-hydroxylation from preparation to preparation of isozyme 6 is not presently understood. Electrophoretic examination of the isozymes suggests that they are homogeneous, and HPLC maps of the isozymes after digestion with *Staphylococcus aureus* V_8 protease (not shown) suggests that they are identical. Further investigation will be required to determine if the differences are significant.

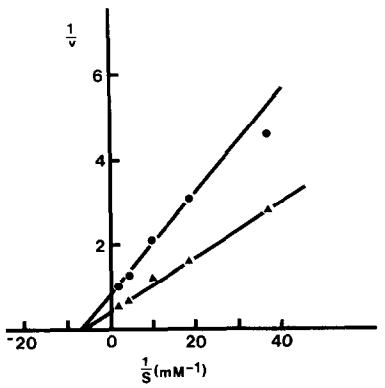


FIG. 6. Lineweaver-Burk plot of PGE_1 metabolism by isozyme 6 from imidazole-treated rabbits. Velocity (v) = nmol product min^{-1} nmol *P*-450^{-1}. ●, 18-OH; ▲, 19-OH.

As previously indicated, 3-methylcholanthrene treatment, which is known to markedly elevate hepatic *P*-450c and to a lesser extent *P*-450d in rats (13), dramatically increases the microsomal ω-2 hydroxylation of PGE_1 and PGE_2 (19), suggesting that in rat liver *P*-450c is primarily responsible for the ω-2 hydroxylation of PGEs.[8] Rabbit isozyme 6 appears to be re-

[8] 3-Methylcholanthrene (MC) treatment of both Sprague-Dawley (S-D) and Long-Evans (L-E) rats

sponsible for the ω-2 hydroxylation in rabbit hepatic microsomes. Thus, the structural similarity of isozyme 6 and *P*-450c indicated by comparison of primary structures (29) and of immunochemical properties (27) apparently accounts for the identical regiospecificity seen in the hydroxylation of PGs. Isozyme 6 is inducible in the neonate (28) but, unlike *P*-450c in the rat, is not markedly induced in the adult rabbits. Thus, the much lower activity in rabbit microsomes is probably a reflection of the low specific content of this isozyme in the adult. It would be of interest to examine neonatal liver after TCDD treatment, where the isozyme is selectively induced (28). ω-2 Hydroxylation of saturated long-chain fatty acids has recently been observed with a soluble *P*-450 monooxygenase from *Bacillus megaterium* ATCC 14581 (15). Whether the bacterial enzyme exhibits structural similarities to isozyme 6 is not yet known.

In conclusion, isozyme 6 differs markedly from other rabbit liver *P*-450 isozymes with respect to PG metabolism. Hydroxylation occurs at ω-1 and ω-2 positions and activity is not increased by cytochrome b_5. Since ω-2-hydroxy metabolites were not detected in the urine, but alkyl side chain dinor-prostaglandin dicarboxylic acids were identified in the urine (17, 18), it is conceivable that these acids are formed by an initial ω-2 hydroxylation followed by an oxidative cleavage of the C_{19}–C_{20} fragment rather than by the classical β oxidation of the ω-carboxy derivative of the alkyl side chain. Isozyme 6 is present in a variety of rabbit tissues; lung, aorta, heart, and bladder (16). Future studies should provide information as to whether these tissues also effectively catalyze the hydroxylation of PGs and whether regioselectivity of hydroxylation, at ω-2 and ω-1, reflects the presence of this isozyme.

yields similarly stimulated ω-2 hydroxylation of PGE_2 (K. Holm and D. Kupfer, unpublished). Since MC induces *P*-450_c in L-E rats (13) and *P*-450_{57K} in S-D rats (14), we propose that the ω-2 hydroxylation of PGEs is catalyzed by the major MC-inducible *P*-450.

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REFERENCES

1. HOLM, K. A., KOOP, D. R., COON, M. J., AND KUPFER, D. (1983) *Fed. Proc.* **42,** Abstr. 3613.
2. KOOP, D. R., KUPFER, D., THEOHARIDES, A. D., VATSIS, K. P., AND COON, M. J. (1983) *in* Proceedings, 74th Ann. Meet. Amer. Oil Chem. Soc., May 8-12.
3. ISRAELSSON, U., HAMBERG, M., AND SAMUELSSON, B. (1969) *Eur. J. Biochem.* **11,** 390-394.
4. SAMUELSSON, B., GRANSTROM, E., GREEN, K., AND HAMBERG, M. (1971) *Ann. N. Y. Acad. Sci.* **180,** 138-163.
5. KUPFER, D. (1982) *in* Hepatic Cytochrome P-450 Monooxygenase System (Schenkman, J. B., and Kupfer, D., eds.), pp. 157-187, Pergamon Oxford.
6. KUPFER, D. (1974) *Life Sci.* **15,** 657-670.
7. KUPFER, D., NAVARRO, J., AND PICCOLO, D. E. (1978) *J. Biol. Chem.* **253,** 2804-2811.
8. POWELL, W. S. (1978) *J. Biol. Chem.* **253,** 6711-6716.
9. KUPFER, D., MIRANDA, G. K., NAVARRO, J., PICCOLO, D. E., AND THEOHARIDES, A. D. (1979) *J. Biol. Chem.* **254,** 10405-10414.
10. THEOHARIDES, A. D., AND KUPFER, D. (1981) *J. Biol. Chem.* **256,** 2168-2175.
11. OKITA, R. T., PARKHILL, L. K., YASUKOCHI, Y., MASTERS, B. S. S., THEOHARIDES, A. D., AND KUPFER, D. (1981) *J. Biol. Chem.* **256,** 5961-5964.
12. VATSIS, K. P., THEOHARIDES, A. D., KUPFER, D., AND COON, M. J. (1982) *J. Biol. Chem.* **257,** 11221-11229.
13. THOMAS, P. E., REIK, L. M., RYAN, D. E., AND LEVIN, W. (1983) *J. Biol. Chem.* **258,** 4590-4598.
14. CHENG, K.-C., KRUTZSCH, H. C., PARK, S. S., GRANTHAM, P. H., GELBOIN, H. V., AND FRIEDMAN, F. K. (1984) *Biochem. Biophys. Res. Commun.* **123,** 1201-1208.
15. KIM, B. H., AND FULCO, A. J. (1983) *Biochem. Biophys. Res. Commun.* **116,** 843-850.
16. PHILPOT, R. M., DOMIN, B. A., SERABJIT-SINGH, C. J., VANDERSLICE, R. R., DEVEREUX, T. R.,

FOUTS, J. R., AND BEND, J. R. (1984) *in* Proceedings, IUPHAR 9th Intl. Congr. Pharmacol., London, Abstr. S26-4.

17. SUN, F. F. (1974) *Biochim. Biophys. Acta* **348,** 249–262.
18. SUN, F. F. (1974) *Biochim. Biophys. Acta* **369,** 95–110.
19. HOLM, K. A., ENGELL, R. A., AND KUPFER, D. (1985) *Arch. Biochem. Biophys.* **237,** 477–489.
20. ANDERSON, W. G., PICCOLO, D. E., AND KUPFER, D. (1979) *in* Biol./Biomed. Applications of Liquid Chromatography II, pp. 416–423, Dekker, New York.
21. COON, M. J., VAN DER HOEVEN, T. A., DAHL, S. B., AND HAUGEN, D. A. (1978) *in* Methods in Enzymology (Fleischer, S., and Packer, L, eds.) Vol. 52, pp. 109–117, Academic Press, New York.
22. KOOP, D. R., AND COON, M. J. (1984) *Mol. Pharmacol.* **25,** 494–501.
23. FRENCH, J. S., AND COON, M. J. (1979) *Arch. Biochem. Biophys.* **195,** 565–577.
24. STRITTMATTER, P., FLEMING, P., CONNORS, M., AND CORCORAN, D. (1978) *in* Methods in Enzymology (Fleischer, S., and Packer, L., eds.) Vol. 52, pp. 97–101, Academic Press, New York.
25. RYAN, D. E., WOOD, A. W., THOMAS, P. E., WALZ, F. G., Jr., YUAN, P.-M., SHIVELY, J. E., AND LEVIN, W. (1982) *Biochim. Biophys. Acta* **709,** 273–283.
26. FUJITA, V. S., BLACK, S. D., TARR, G. E., KOOP, D. R., AND COON, M. J. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 4260–4264.
27. THOMAS, P. E., REIDY, J., REIK, L. M., RYAN, D. E., KOOP, D. R., AND LEVIN, W. (1984) *Arch. Biochem. Biophys.* **235,** 239–253.
28. NORMAN, R. L., JOHNSON, E. F., AND MULLER-EBERHARD, U. (1978) *J. Biol. Chem.* **253,** 8640–8647.
29. BLACK, S. D., AND COON, M. J. (1985) *in* Cytochrome P-450: Structure, Mechanism, and Biochemistry (Ortiz de Montellano, P. R., ed.), Plenum, New York, in press.
30. HOLM, K. A., AND KUPFER, D. (1985) *J. Biol. Chem.* **260,** 2027–2030.
31. GORSKY, L. D., AND COON, M. J. (1985) *Fed. Proc.* **44,** 1611.