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Bromobenzene Metabolism in the Rabbit

Specific Forms of Cytochrome P-450 Involved in 2,3- and 3,4-Epoxidation

SERRINE S. LAU AND VINCENT G. ZANNONI

Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Michigan 48109

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SUMMARY

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Previous studies in our laboratory indicated that phenobarbital treatment of rats caused a significant increase in both 2,3- and 3,4-epoxidation of bromobenzene in their hepatic microsomes and that 3-methylcholanthrene or β-naphthoflavone caused a selective increase in the 2,3-epoxidation pathway. Sodium dodecyl sulfate, polyacrylamide gel electrophoresis of microsomes revealed multiple forms of cytochrome P-450, in keeping with the notion that different species of the heme protein catalyzed the "nontoxic" 2,3-epoxidation and the "toxic" 3,4-epoxidation of this environmental chemical. The present study describes the metabolism of bromobenzene with highly purified cytochrome P-450 and P-448 isolated from rabbit hepatic microsomal preparations. This study involved the enzymatic conversion of bromobenzene to *o*-bromophenol via 2,3-epoxidation and *p*-bromophenol via 3,4-epoxidation in a reconstituted mixed-function oxygenase system. Evidence is presented that purified rabbit cytochrome P-450 (LM_2) prepared from animals treated with phenobarbital specifically catalyzes the 3,4-epoxidation of bromobenzene to *p*-bromophenol. Furthermore, evidence is given that purified rabbit cytochrome P-448 (LM_4) prepared from animals treated with β-naphthoflavone specifically catalyzes the 2,3-epoxidation of bromobenzene to *o*-bromophenol. These data represent an interesting example of two epoxidation pathways involved in the metabolism of a common substrate, one of which leads to cellular damage, i.e., phenobarbital-inducible 3,4-epoxidation; the other, i.e., β-naphthoflavone-inducible 2,3-epoxidation of bromobenzene, is not particularly detrimental. Each epoxidation pathway preferentially requires a different and specific form of the heme protein.

The cytotoxicity due to the metabolism of bromobenzene relies mainly on the conversion of this environmental chemical via its 3,4-epoxidation to *p*-bromophenol (1–4). In addition, bromobenzene undergoes 2,3-epoxidation to *o*-bromophenol, which is much less detrimental to the cells (2, 4–6). Our previous studies have demonstrated specificity of the cytochrome P-450 mixed-function oxygenase system in catalyzing either 3,4- or 2,3-epoxidation pathways of bromobenzene (7). Induction studies with phenobarbital, 3-methylcholantrene, and β-naphthoflavone as well as sodium dodecyl sulfate gel electrophoresis gave credence to the involvement of specific forms of cytochrome P-450 responsible for these pathways (7). Previous studies by Mitchell and co-workers (8) demonstrated that a variety of species, including mice, rats, hamsters, and rabbits, metabolized bromobenzene via epoxides leading to varying degrees of hepatic centrolobular necrosis. The present communication presents data on the 2,3- and 3,4-epoxidation of bromobenzene with a reconstituted mixed-function oxygenase system using preparations of highly purified cytochrome P-450 (LM_2; mol wt, 48,700) prepared from cells of phenobarbital-treated rabbits. In addition, highly purified cytochrome P-448 (LM_4; mol wt, 55,300) prepared from β-naphthoflavone-treated rabbits was used.

The enzymatic assay for hepatic microsomal epoxidation of bromobenzene was carried out as described by Lau and Zannoni (7). The products of the reaction, namely *o*-bromophenol and *p*-bromophenol, were identified by gas chromatography with electron capture detection as described by Lau and Zannoni (7). Purified forms of rabbit cytochrome P-450, namely LM_2 and LM_4 prepared by polyethylene glycol fractionation, DEAE-cellulose, and hydroxylapatite/silica gel chromatography, were obtained according to the methods of Haugen

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TABLE 1

Enzymatic conversion of bromobenzene to o-bromophenol and p-bromophenol with purified rabbit cytochrome P-450[a] with a reconstituted system[b]

	Specific activity	
	o-Bromophenol	*p*-Bromophenol
	nmoles/10 min/nmole purified P-450 at 37°	
LM_2	ND[c]	10.0
LM_4	1.6	ND[c]

[a] Cytochrome P-450 was purified from phenobarbital- or 3-methylcholanthrene-treated rabbits as described previously (9).

[b] The reconstituted incubation system contained 0.1 nmole of cytochrome P-450 LM_2 or P-450 LM_4, 0.1 nmole of NADPH-cytochrome *c* reductase, and 0.06 mg of dilauroylglyceryl-3-phosphorylcholine in a total incubation volume of 1.2 ml. Bromobenzene (3.0 μmoles) was incubated in the presence of NADPH (1.0 μmole) at 37°c for 10 min. The constituents were added in excess; the rate of the reaction was linear with time and proportional to enzyme concentration (11).

[c] ND, Nondetectable.

and Coon (9). Both fractions containing cytochrome P-450 are highly purified; LM_2, 18.0 nmoles/mg; LM_4, 15.0 nmoles/mg (>90% according to theoretical calculations). In addition, rabbit NADPH cytochrome P-450 reductase was obtained with a purity of 11.7 nmoles/mg (10). The assay conditions in the reconstitution experiments are described in the legend to Table 1.

The results of the enzymatic conversion of bromobenzene to *o*-bromophenol via 2,3-epoxidation and *p*-bromophenol via 3,4-epoxidation with highly purified cytochrome P-450 preparations in a reconstituted incubation system are given in Table 1. Cytochrome P-450 induced with phenobarbital (LM_2; mol wt, 48,700) specifically catalyzed only the 3,4-epoxidation pathway to *p*-bromophenol, 10 nmoles/10 min/nmole of purified cytochrome P-450. In contrast, cytochrome P-448 (LM_4; mol wt, 55,300) induced by treatment of animals with β-naphthoflavone selectively catalyzed only the 2,3-epoxidation to *o*-bromophenol, 1.6 nmoles/10 min/nmole of purified cytochrome P-448. In addition, the ratio of the specific activities of the quantity of *p*-bromophenol/*o*-bromophenol with purified cytochrome P-450 preparations (in the order of 5:1) is in close agreement with the ratio of these epoxidation pathways using crude rabbit microsomes for the incubation; the specific activity of *p*-bromophenol formation is 45.1 and that of *o*-bromophenol formation is 11.0 nmoles/min/100 mg of microsomal protein. These results conclusively indicate that the multimetabolic pathways of bromobenzene, i.e., 2,3- and 3,4-epoxidation, are catalyzed by specific forms of the heme protein. It is also of interest that one of the epoxidation pathways, namely 3,4-epoxidation, has been shown to be more cytotoxic in rats and mice (2, 4). Furthermore, cytotoxicity *in vivo* has been shown to occur in the rabbit (8), and an examination of the urinary metabolites after bromobenzene administration indicated the presence of *p*-bromophenol (12).

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Send reprint requests to: Dr. Vincent G. Zannoni, Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Mich. 48109.