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SELECTIVITY OF 1-PHENYLIMIDAZOLE AS A LIGAND FOR CYTOCHROME P-450 AND AS AN INHIBITOR OF MICROSOMAL OXIDATION

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Abstract—Equilibrium dialysis studies established that 1-[4′-(^{3}H)-phenyl]imidazole (PI) was bound to hepatic microsomal suspensions from control, phenobarbital (PB)- and 3-methylcholanthrene (3MC)-treated rats and that the binding was directly related to the cytochrome P-450 content. Computer-assisted Scatchard plot analysis of the binding data indicated the existence of two major types of microsomal binding sites in both control and induced rats, one with a high affinity ($K_a \sim 1.5 \times 10^7\,M^{-1}$) and the other with a low affinity ($K_a \sim 5 \times 10^5\,M^{-1}$) for PI. The binding of PI to the highly purified, individual cytochrome P-450s that constituted the major forms from the PB- and β-naphthoflavone (βNF)-induced rats exhibited affinities similar to the high and low affinity binding sites observed in microsomal suspensions. The two types of PI binding sites were characteristic of two classes of cytochrome P-450, and the major cytochrome induced by PB and 3MC (or βNF) were each associated with one of these two classes. In concurrence with this, it was shown that, although PI was an excellent inhibitor of aromatic hydrocarbon hydroxylase (AHH) activity in PB-induced rats, it exhibited little or no inhibitory activity towards AHH activity in 3MC-induced animals.

It is now well established that a variety of compounds representing several classes of nitrogen-containing heterocycles, such as imidazoles [1–8], benzimidazoles [9], oxazoles and thiazoles [10], 1,2,3-benzothiadiazoles [11–13], ellipticines [14–16] and pyrroles [17], are effective inhibitors of microsomal drug oxidations. The 1- and 4(5)-substituted arylimidazoles, initially reported to be inhibitors of steroid 11β-hydroxylation [18], are among the most potent *in vitro* inhibitors of cytochrome P-450-mediated drug oxidations; *in vivo* they prolong barbiturate sleeping time in mammals [4, 6, 8] and synergize insecticide toxicity to houseflies [5].

As a result of apparent relationships between inhibitory potency and spectral (Type II) dissociation constants (K_s) of a number of 1- and 4(5)-substituted imidazoles, it was proposed that inhibition by these compounds results primarily from coordination of the nonbonded electrons at N-3 of the imidazole ring with the 5th or 6th ligand of the heme iron of cytochrome P-450 [6]. This proposal was supported by the results of structure–activity studies clearly showing that both binding and inhibition are dependent on the presence of a sterically unhindered nitrogen atom at position-3 of the imidazole ring [19]. The demonstration that, in a series of 1-alkylimidazoles, inhibitory potencies and K_s values are optimal in compounds containing 8–10 carbon atoms is indicative of the importance of additional hydrophobic interactions in the vicinity of the heme moiety [7]. Further evidence for this has been provided by the successful use of 1-(4-azidophenyl)imidazole as a photoaffinity probe for characterization of the active site of cytochrome P-450$_{cam}$ [20].

The results of early studies, conducted with a limited number of *in vitro* assays, suggested that the 1-arylimidazoles exhibit a similar degree of inhibitory potency towards most oxidative reactions. It was subsequently observed, however, that 1-phenylimidazole was surprisingly inactive in some assays, particularly towards benzo[*a*]pyrene hydroxylase activity in microsomes from 3-methylcholanthrene-induced rats.† This observation suggested that, as has been reported previously for the ellipiticines [16, 21], the 1-arylimidazoles might interact differently with different forms of cytochrome P-450 and that consequently they might constitute useful probes for studying the heterogeneity of the multiple forms of cytochrome P-450 now known to exist [22–24].

This paper reports the results of studies on the binding of 1-[4′-(^{3}H)-phenyl]imidazole to microsomes from control, phenobarbital-, and 3-methylcholanthrene-treated rats, using the technique of equilibrium dialysis.

MATERIALS AND METHODS

Chemicals. The 1-[4′-(^{3}H)-phenyl]imidazole (PI) employed in this study was prepared by the Amersham Corp., Arlington Heights, IL, by reductive tritiation of 1-(4′-bromophenyl)imidazole syn-

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† C. F. Wilkinson, unpublished observation.

thesized in this laboratory as previously described [18]. A portion of the radioactive material was diluted with cold PI, and the mixture was purified on a silicic acid column using first benzene and subsequently acetone as elutants. Fractions containing radioactivity were subjected to thin-layer chromatography on silica gel plates (F-254), and those with autoradiographic peaks cochromatographing with purified cold PI were combined. Chemical and radiochemical purity and identity of the combined fractions with PI were established by thin-layer chromatography in five different solvent mixtures: benzene (R_f 0.03), benzene–acetone (4:1) (R_f 0.31), benzene–acetone (2:3) (R_f 0.56), ethyl acetate–acetone (2:1) (R_f 0.59) and ethyl acetate saturated with acetic acid (R_f 0.46). Specific activity of the purified radiolabeled PI was 14.9 mCi/mmole.

Biochemicals were purchased from the Sigma Chemical Co., St. Louis, MO, and all other chemicals and solvents employed were of analytical reagent grade.

Animals and microsome preparation. Male Sprague–Dawley derived rats (200–300 g) were purchased from Blue Spruce Farms, Altamont, NY. Phenobarbital (PB) was given i.p. in 0.9% saline at a dose of 100 mg/kg once daily for 3 days; control animals received 0.9% saline solution. 3-Methylcholanthrene (3MC) dissolved in corn oil was given i.p. at a dose of 50 mg/kg once daily for 3 days; control animals were given corn oil alone.

Hepatic microsomal fractions were prepared, as previously described [25], from control, PB-, or 3MC-treated animals. Microsomes were stored as frozen dry pellets until required but were not used for binding studies after more than 2 weeks of storage. Protein was determined by the method of Lowry *et al.* [26].

Cytochrome P-450 preparation. Cytochrome P-450 was purified as previously described [27] by *n*-octylamino Sepharose 4B and DEAE cellulose chromatography from detergent-solubilized hepatic microsomes of rats treated with either PB or β-naphthoflavone (βNF). Binding was studied using the B_2-fraction, which in each case was electrophoretically homogenous and represented the major form of the cytochrome in either PB- or βNF-induced microsomes.

Equilibrium dialysis. Dialysis was conducted with an Equilibrium Dialyzer (Spectrum Medical Industries Inc., Los Angeles, CA) equipped with 20 Teflon semimicro cells, each consisting of two half-cells with a working volume of 1.0 ml separated by a 4.5 cm^2 area of Spectrapor 2 dialysis membrane (Spectrum Medical Industries Inc.). After assembling the cells in their holders, one half of each cell received 1.0 ml of the appropriate microsomal suspension (about 2 mg protein/ml) in 50 mM Tris–HCl buffer, pH 7.6, containing 1 mM EDTA and the other half 1.0 ml of the desired concentration (range 1.0×10^{-7} M to 1.3×10^{-5} M) of radiolabeled PI in the same buffer. The holders containing the cells were placed on the dialyzer drive unit and rotated at 15 rpm for 90 min at room temperature until equilibrium was attained. Triplicate 0.1-ml aliquots of the contents of each half-cell were then removed, mixed with 10 ml liquiscint (National Diagnostics, Somerville, NJ) and counted in a model 2425 Packard Tri-Carb Liquid Scintillation Counter. Counting efficiency was about 47%, and preliminary studies indicated that quenching was not a problem at the protein concentrations employed. Following correction for background, the amount of PI bound to the microsomes (B) was calculated from the difference in counts between the half-cell containing the microsomal suspension ($B + L$) and that containing the free ligand (L); B may be expressed as nmoles of PI bound per ml of microsomal suspension, per mg microsomal protein or per nmole of cytochrome P-450 as measured spectrophotometrically.

Analysis of binding data. Data were analyzed on the DEC 20 computer at Cornell University utilizing the LIGAND program of Munson and Rodbard [28]. LIGAND is a non-linear model fitting program that provides optimal (weighted least squares) estimates of affinity constants and binding capacities. The data employed for the analyses were pooled from at least five different microsomal suspensions prepared from either control or appropriately induced animals over a period of about 6 months. Non-specific binding was measured with denatured rat liver microsomes in which cytochrome P-450 had been totally converted to cytochrome P-420 (indicated spectrophotometrically) by warming the microsomal suspension (2 mg protein/ml) for 30 min at 60°.

Other assays. Cytochrome P-450 was measured by the method of Omura and Sato [29] using an Aminco Chance DW 2 spectrophotometer and employing an extinction coefficient of 91 mM^{-1} cm^{-1} for the cytochrome P-450-ferrous carbonyl spectral complex.

Aromatic hydrocarbon (benzo[*a*]pyrene) hydroxylase (AHH) activity was determined fluorimetrically by the method of Yang and Kicha [30] using an Aminco SPF-125 spectrophotofluorimeter.

RESULTS AND DISCUSSION

Preliminary studies indicated that total binding (B) of PI to microsomal suspensions increased with time of dialysis up to about 60 min, when equilibrium had been attained, and increased linearly with microsomal protein concentration up to about 3 mg protein/ml. In either 50 mM phosphate or 50 mM Tris–HCl buffer containing 1 mM EDTA, binding increased with increasing pH from 6.0 to 7.0 and thereafter remained constant to pH 9.0; at pH 7.6, the pH selected for routine binding assay, total binding was not influenced by the type of buffer employed.

Binding of PI to hepatic microsomal suspensions (2 mg protein/ml) from untreated or control (saline or corn oil treated) rats increased with increasing ligand concentration over the range 1.0×10^{-7} M to 1.0×10^{-5} M and plots of B (nmoles PI bound/nmole of cytochrome P-450) versus L (free PI concentration, μM) exhibited saturation characteristics when corrected for non-specific binding (Fig. 1). The non-specific binding that was measured in gently denatured (30 min at 60°) microsomes in which all cytochrome P-450 had been totally converted to cytochrome P-420 increased linearly with increasing ligand concentration. The dramatic decrease in PI binding in denatured microsomes and the subse-

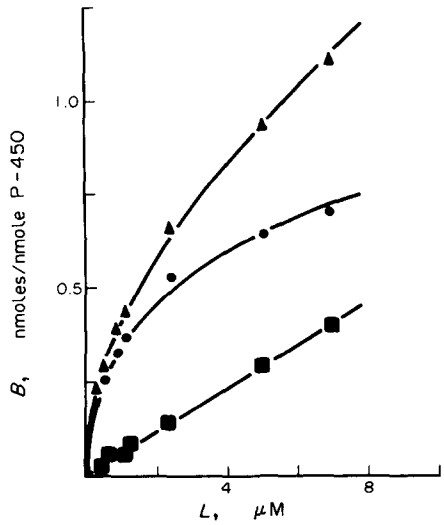

Fig. 1. Effect of ligand concentration (L) on the binding (B) of PI to hepatic microsomal suspensions from control rats. Plots shown are total binding (nmoles PI/nmole cytochrome P-450) (▲—▲), non-specific binding to an equivalent suspension of microsomes in which cytochrome P-450 has been converted to cytochrome P-420 (■—■), and specific binding (nmoles PI/nmole cytochrome P-450) (●—●) obtained by subtracting non-specific from total binding. Experimental details are described in Materials and Methods.

quently confirmed failure of PI to bind to cytochrome P-420 formed by denaturation of purified cytochrome P-450 strongly suggested that PI binding was associated with cytochrome P-450. Furthermore, the data in Fig. 1 indicate that the total amount of PI bound approached the total concentration of cytochrome P-450 as determined spectrophotometrically and suggested that PI binding to the cytochrome might occur in a 1:1 molar ratio.

Double-reciprocal plots ($1/B$ vs $1/L$) from the same group of data clearly deviated from linearity (Fig. 2) and suggested the presence of at least two types of binding sites with distinct binding characteristics.

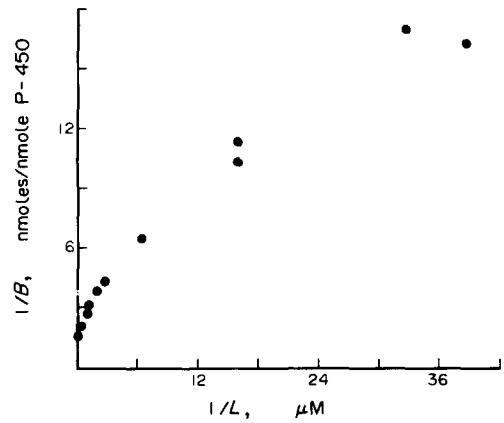

Fig. 2. Lineweaver–Burk reciprocal plot of specific PI binding to hepatic microsomal suspensions from control rats, using data from the same group as in Fig. 1.

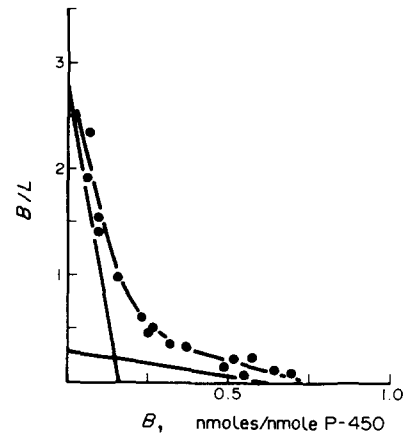

Fig. 3. Scatchard plot of data in Fig. 1. The solid circles are representative (from a total of 95) data points corrected for non-specific binding and the lines are drawn from computer-obtained data for the high affinity (steep slope) and low affinity (shallow slope) sites.

This was supported by the corresponding Scatchard plot (B/L vs B) which was non-linear and asymptotically approached both the x and y axes (Fig. 3).

Quantitative examination of the data using the LIGAND computer program [28] indicated a good fit to a two-site binding model and provided affinities (K_1 and K_2) and relative capacities (concentrations) (R_1 and R_2) of both the high and low affinity sites respectively. The data in Table 1 show that in control microsomes the affinity of the high affinity sites for PI ($K_1 = 1.7 \pm 0.7 \times 10^7\,M^{-1}$) was approximately 37-fold that of the low affinity site ($K_2 = 4.6 \pm 2.1 \times 10^5\,M^{-1}$). Consequently, if, as previously suggested, the binding of PI is specific for cytochrome P-450, the untreated rat liver microsomes appear to contain two distinct subpopulations of cytochrome P-450 that exhibit a 37-fold difference with respect to their affinities for PI.

Data obtained from the LIGAND program (Table 1) indicate that the total concentration of the low and high affinity sites ($R_1 + R_2$) in the untreated rat liver microsomes accounted for approximately 76% of the total cytochrome P-450, assuming that it binds to PI in a 1:1 molar ratio. The apparent failure to account for almost 25% of the cytochrome P-450 in the microsomes may indicate that the assumed 1:1 PI:cytochrome P-450 binding ratio is incorrect and, indeed, there is some evidence (see later discussion) that this may be the case with at least some forms of cytochrome P-450. The discrepancy could also be caused by a relatively small error in measuring non-specific binding or inaccuracy in the spectrophotometric estimation of the cytochrome P-450-ferrous carbonyl spectral complex [29].

If, for the purposes of this discussion, it is assumed that PI binds specifically to cytochrome P-450 in a 1:1 molar ratio, and if the cytochrome P-450 to which binding was observed is fully representative of that present in the microsomes, the subpopulation of cytochrome P-450 with a high affinity for PI ($R_1 = 0.16 \pm 0.04$) constituted 21% of the total ($R_1 + R_2 = 0.76$) and the subpopulation with a low

Table 1. Characteristics of binding of 1-[4′-(^{3}H)-phenyl]imidazole to hepatic microsomes from control, phenobarbital- and 3-methylcholanthrene-induced rats*

	High affinity site		Low affinity site		Total binding
	K_1 (M^{-1})	R_1 (nmoles PI/nmole cytochrome P-450)	K_2 (M^{-1})	R_2 (nmoles PI/nmole cytochrome P-450)	$R_1 + R_2$ (nmoles PI/nmole cytochrome P-450)
Control	$1.71 \pm 0.69 \times 10^7$	0.16 ± 0.04	$4.63 \pm 2.12 \times 10^5$	0.60 ± 0.08	0.76
PB-induced	$1.20 \pm 0.60 \times 10^7$	0.37 ± 0.06	$5.78 \pm 3.58 \times 10^5$	0.36 ± 0.05	0.73
3MC-induced	$0.91 \pm 0.41 \times 10^7$	0.13 ± 0.06	$5.12 \pm 1.96 \times 10^5$	0.68 ± 0.06	0.81

* Values for K and R were obtained from the LIGAND computer program [28] from analysis of 95 (control), 52 (phenobarbital) and 53 (3-methylcholanthrene) separate data points, respectively, each representing the mean of duplicate or triplicate binding experiments conducted over several months. Values are means ± S.D.

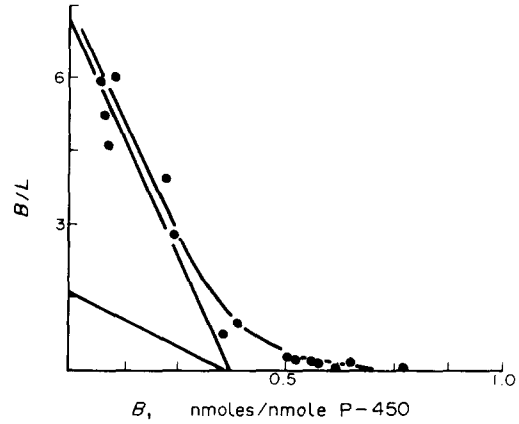

Fig. 4. Scatchard plot of PI binding to hepatic microsomes from PB-induced rats. Data points are representative of a total of 52 used in the analysis. See legend to Fig. 3 for explanation.

affinity for PI ($R_2 = 0.60 \pm 0.08$) constituted 79% of the total.

Although, because of enhanced levels of cytochrome P-450, the actual amount of PI bound to hepatic microsomal suspensions from PB- and 3MC-induced rats was greater than in untreated animals (or corn oil or saline treated controls), qualitatively similar binding characteristics were observed. Scatchard plots (Figs. 4 and 5) were non-linear and in each case the LIGAND program indicated a good fit to a two-site binding model similar to that previously described. As indicated in Table 1, the affinities of the high and low affinity sites for PI binding were quite similar in both PB- and 3MC-induced microsomes and were not significantly different from the values obtained with controls. Values for total specific binding capacity ($R_1 + R_2$), expressed in terms of nmoles PI bound per nmole of cytochrome P-450, were also quite similar in induced and control microsomes. Thus, assuming equimolar binding between PI and cytochrome P-450 (i.e. a theoretical maximum specific binding of 1.0), total PI binding capacity accounted

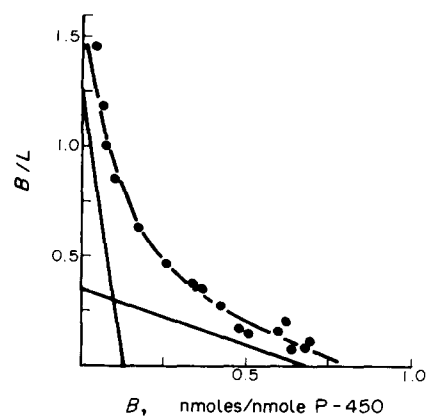

Fig. 5. Scatchard plot of PI binding to hepatic microsomes from 3MC-induced rats. Data are representative of a total of 53 used in the analysis. See legend to Fig. 3 for explanation.

for 73 and 81%, respectively, of the spectrophotometrically determined cytochrome P-450 in PB- and 3MC-induced microsomes.

However, the relative contributions of the high and low affinity sites to the total binding capacity were quite different in microsomes from PB- and 3MC-induced animals and these, in turn, were different from those measured in control microsomes. Thus, in microsomes from PB-induced rats, the high affinity site was responsible for about 51% of the total binding (compared with 21% in controls) and the low affinity site accounted for about 49% of the total (compared with 79% in controls). In contrast, the percentages of the high and low affinity sites in microsomes from 3MC-induced rats were 16% and 84%, respectively, which was quite similar to their percentages in control microsomes. More meaningful information is revealed when the percentages of the high and low affinity sites are expressed in terms of the actual amounts of cytochrome P-450 that they represent. The data in Table 2 clearly show that, relative to controls, induction with PB was associated with a 4.4-fold increase in the amount of the high affinity PI-binding form, with little or no increase in the low affinity form. In contrast, induction with 3MC had no apparent effect on the level of the high affinity form but caused a 1.6-fold increase in the concentration of the low affinity form above control levels.

As a result of intensive research efforts in recent years, there is now ample evidence for the existence of multiple forms of hepatic cytochrome P-450 in a variety of mammalian species [22–24]. Although the precise number of different forms remains unknown and is still a matter for some conjecture, catalytically, structurally, and immunochemically distinct groups of cytochrome P-450 can be identified following induction of animals with PB or 3MC; these have commonly been referred to by the generic terms cytochrome P-450 and cytochrome P-448, respectively, and their syntheses appear to be under separate genetic control [23, 24]. Thus, using genetically inbred strains of mice, it has been shown that the so-called cytochrome P-448, inducible by 3MC, β-naphthoflavone (βNF) or 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, and associated primarily with the metabolism of polycyclic aromatic hydrocarbons, is in fact a group of cytochromes, the induction of which is regulated by a single gene locus, the Ah locus [31]. Synthesis of the cytochrome P-450 induced by PB is not mediated by the Ah locus.

In view of the specific enrichment of microsomes with the high and low affinity PI binding sites following treatment of rats with PB and 3MC, respectively (Table 2), it was of considerable interest to measure PI binding to individual purified cytochromes representative of those induced by PB and 3MC. Scatchard plots of PI binding to the purified, electrophoretically homogeneous B_2 fractions from PB- and βNF-induced rat liver microsomes [27] (Fig. 6) were linear and, in each case, a good fit of the data to a single-site model was confirmed by the LIGAND program. It should be pointed out that the properties of the B_2 fractions from 3MC- and βNF-induced rats are identical [27]. The affinity of PI for the purified PB-B_2 cytochromes P-450 was $4.48 \pm 0.41 \times 10^6\,M^{-1}$ and that for the purified βNF-B_2 hemoprotein was $6.53 \pm 0.63 \times 10^5\,M^{-1}$. This latter value is very similar to that observed for the low affinity site measured in intact microsomes (Table 1); the affinity value for the PB-B_2 cytochrome P-450 was 2- to 3-fold lower than that of the high affinity site in intact microsomes. However, it has been subsequently observed that affinity values obtained with the purified cytochrome are subject to modification dependent on the amount of detergent present in the preparations (detergent decreases affinity), and it is probable that this will differ somewhat in different preparations. Thus, a higher affinity value of $8.27 \times 10^6\,M^{-1}$ was obtained recently with a PB-B_2 cytochrome P-450 fraction subjected to more extensive removal of detergent.

The total binding capacities (R) of the purified cytochromes are also interesting (Figs. 6 and 7). In the case of the PB-B_2 hemoprotein (Figs. 6 and 7) the total binding capacity was 0.87 ± 0.03 nmole PI/nmole of cytochrome P-450 and was close to the 1:1 molar ratio previously discussed. With the purified βNF-B_2 hemoprotein, however, a value of only 0.54 ± 0.03 nmole PI/nmole of cytochrome P-450

Table 2. Concentrations of low and high affinity PI binding sites expressed in terms of cytochrome P-450 concentration

Treatment	Cytochrome P-450 (nmoles/mg protein)		
	Total	High affinity site	Low affinity site
Corn oil or saline control	0.70 ± 0.07*	0.15 (21)†	0.55 (79)
Phenobarbital (100 mg/kg × 3)	1.30 ± 0.09	0.66 (51)	0.64 (49)
3-Methylcholanthrene (50 mg/kg × 3)	1.07 ± 0.13	0.17 (16)	0.90 (84)

* Means ± S.D.

† Values in parentheses are percent concentrations of the high and low affinity sites based on the total number of binding sites ($R_1 + R_2$) obtained from the LIGAND program (see Table 1). The calculations are based on the assumption of a 1:1 PI:cytochrome P-450 binding ratio.

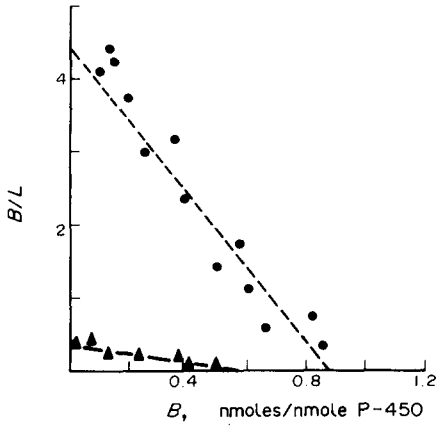

Fig. 6. Scatchard plots of PI binding to the purified B_2 fraction from PB-induced rats (●—●) and to the B_2 fraction from βNF-induced rats (▲—▲). For explanation see text.

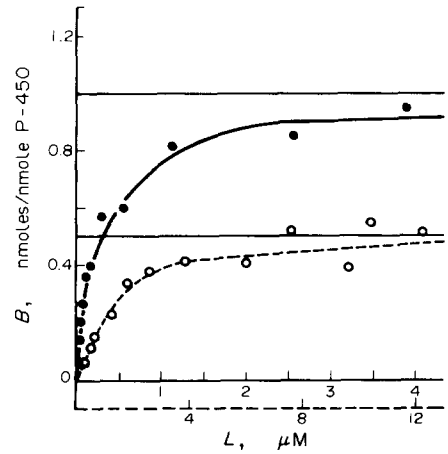

Fig. 7. Effect of ligand concentration (L) on binding (B) of PI to purified PB-B_2 (solid line) and βNF-B_2 (broken line) cytochrome P-450s. For explanation see text.

was observed (Figs. 6 and 7). An explanation of why the purified βNF-B_2 hemoprotein bound PI in an apparent 2:1 ratio is not immediately obvious; it may have been related to the presence of excessive detergent in the purified preparation or to a difference in the micelle structure (possibly a different pattern of aggregation) that in some way precluded the expected equimolar binding. If, on the other hand, some portion of the cytochrome P-450 present in microsomes does indeed bind to PI in a 2:1 molar ratio, this could provide another explanation for the failure of $R_1 + R_2$ (Table 1) to account for 100% of the total cytochrome P-450 measured spectrophotometrically. The only way in which the problem can be resolved satisfactorily is to conduct PI binding studies on each individual purified form of cytochrome P-450. Unfortunately, the absence of precise information on PI–cytochrome P-450 binding ratios detracts from the significance of quantitative estimations of the relative concentrations of cytochrome P-450 based on PI binding to each of the two major sites.

The results of these studies indicate that hepatic microsomal suspensions from control, PB- and 3MC-induced rats contain two distinct subpopulations of cytochrome P-450 that can be differentiated by their abilities to undergo ligand interaction with PI. However, since it is now well established that there are more than two forms of cytochrome P-450 in hepatic microsomes [22–24] and since the major forms isolated from PB- and 3MC-induced rats constitute only a small (less than 10%) fraction of the total cytochrome P-450 content in untreated animals [32–34], the purified cytochromes employed in this study must simply constitute examples representative of each of two major subpopulations of hemoproteins. It is important to emphasize that the low and high affinity subpopulations of cytochrome P-450, distinguished by their differing affinities for PI, should not be confused with the different catalytic or structural forms of the cytochrome. It would be of considerable interest, however, if specific cytochromes could be characterized with respect to PI binding as more purified forms become available.

An important implication of the data reported here is the possibility that PI might represent a useful selective inhibitor for the characterization of cytochrome P-450 in intact microsomal suspensions. Although PI and related 1-arylimidazoles are well established inhibitors of several cytochrome P-450-mediated drug oxidations [1–8], it would be anticipated that inhibitory potencies towards different

Table 3. Effects of PI and αNF on AHH activity in hepatic microsomes from control, PB- and 3MC-treated rats

Treatment*	Inhibitor	AHH activity (nmoles/min/mg)	% Control
Control	None	0.85 ± 0.22†	100
Control	PI (100 μM)	0.56 ± 0.02	66
Control	αNF (10 μM)	1.32 ± 0.29	155
PB-induced	None	2.41 ± 0.11	100
PB-induced	PI (100 μM)	0.30 ± 0.17	13
PB-induced	αNF (10 μM)	5.21 ± 0.34	216
3MC-induced	None	8.51 ± 0.17	100
3MC-induced	PI (100 μM)	10.54 ± 0.11	124
3MC-induced	αNF (10 μM)	1.85 ± 0.02	22

* Treatment of animals is described in Materials and Methods.
† Means ± S.D.

types of reactions would vary in a manner reflecting the observed differences in PI binding to the cytochromes in the high and low affinity groups. More specifically, it would be anticipated that the inhibitory potency of PI would be considerably lower toward reactions catalyzed by the cytochromes comprising the low affinity group, that includes the cytochrome P-448 induced by βNF or 3MC. The results in Table 3 show the inhibitory activity of PI towards AHH (benzo[*a*]pyrene hydroxylase) activity in microsomes from control, PB- and 3MC-treated rats; it also includes data for α-naphthoflavone (αNF), an inhibitor with established selectivity towards reactions catalyzed by cytochrome P-448 [35]. In microsomes from PB-treated rats, PI (100 μM) caused almost 90% inhibition of the induced AHH activity, whilst αNF (10 μM) led to a 2-fold stimulation. In contrast, in microsomes from 3MC-induced animals, αNF (10 μM) caused 80% inhibition and PI (100 μM) resulted in a 20% stimulation. These data quite clearly suggest that AHH activity can be induced by either PB or 3MC, but that these reactions are catalyzed by cytochromes in the high and low PI affinity groups, respectively; only that catalyzed by the high affinity, PB-induced cytochrome P-450 was susceptible to inhibition by PI.

The ability of PI to bind selectively to different subpopulations of cytochrome P-450 and, as a result, to exhibit selectivity with respect to inhibition of reactions catalyzed by these cytochromes is of considerable interest and strongly suggests the existence of structural differences at the active site. As has been pointed out by Delaforge *et al.* [21], the fact that cytochrome P-448 catalyzes reactions in sterically-hindered, bay-region positions of relatively large polycyclic molecules suggests that it has a somewhat larger active center than cytochrome P-450. The ability of the ellipticines [16, 21] and αNF [35] to selectively inhibit cytochrome P-448-catalyzed reactions may result, in part, from the relatively large molecular dimensions of these compounds. Further support for this lies in the fact that, although PI itself is apparently unable to meet the steric requirements for inhibition of cytochrome P-448, another imidazole, clotrimazole [1-(*o*-chlorophenyldiphenylmethyl)imidazole], with a considerably larger steric bulk in the 1-position, is reportedly an effective inhibitor of AHH activity [36].

A continuing need exists for the discovery of compounds that, through selectivity as either substrates or inhibitors, provide information on the nature and distribution of different subpopulations of cytochrome P-450 in intact microsomal suspensions. The results reported in this paper suggest that PI might constitute a useful compound for this purpose. Further studies are in progress to more firmly establish the selectivity of PI and to investigate the application of these findings to cytochrome P-450s in other species.

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