Interaction of Polycyclic Aromatic Hydrocarbons and Flavones with Cytochromes P450 in the Endoplasmic Reticulum: Effect on CO Binding Kinetics

Aditya P. Koley,[‡] Richard C. Robinson,[‡] Allen Markowitz,[§] and Fred K. Friedman*,[‡]

Laboratory of Molecular Carcinogenesis, National Cancer Institute, and Biomedical Instrumentation and Engineering Program, National Institutes of Health, Bethesda, Maryland 20892

Received August 29, 1994; Revised Manuscript Received October 20, 1994[⊗]

ABSTRACT: The flash photolysis technique was used to examine the kinetics of CO binding to cytochromes P450 in rat liver microsomes. The effect of polycyclic aromatic hydrocarbons (PAHs) and flavones was used to distinguish the kinetic behavior of the PAH-metabolizing P450 1A1 from that of the remaining multiple microsomal P450s. Applying this approach to microsomes from 3-methylcholanthrene-treated rats showed that although all tested PAHs accelerated CO binding to P450 1A1, the extent varied markedly for different PAHs. The tricyclic PAHs phenanthrene and anthracene enhanced CO binding by 37- and 49-fold, respectively, while several tetracyclic and pentacyclic PAHs increased the rate by 3−16-fold. The results indicate that PAHs exert a dual effect on the rate of CO binding to P450 1A1: a general enhancement via widening of the CO access channel and a reduction that is dependent on PAH size. Although 5,6-benzoflavone increased the rate of CO binding to P450 1A1 by 3.5-fold, it additionally decelerated binding to a constitutive P450 by 15-fold. This flavone thus exerts markedly different effects on two P450s within the same microsomal sample. In contrast, the sole effect of 7,8-benzoflavone was acceleration of CO binding to P450 1A1 by 18-fold. The divergent effects of these isomeric flavones, which only differ in positioning of an aromatic ring, illustrate the sensitivity of CO binding to substrate structure. The varying effects of these PAHs and flavones on CO binding kinetics show that they differentially modulate P450 conformation and access of ligands to the P450 heme and demonstrate that binding of carcinogens to a specific target P450 can be evaluated in its native microsomal milieu.

The cytochromes P450 are a family of hemeprotein enzymes that catalyze the oxidation of a wide variety of lipophilic compounds. These include xenobiotics such as drugs and carcinogens as well as endogenous compounds such as steroids and prostaglandins (Lu & West, 1980; Ortiz de Montellano et al., 1986; Ryan & Levin, 1990). The different forms of P450[1] exhibit unique catalytic activity profiles toward various substrates. In particular, the 3-methylcholanthrene (MC) inducible 1A1 form, which is the major P450 in the livers of rats treated with MC (Thomas et al., 1981; Dannan et al., 1983), has been extensively studied. This P450 efficiently metabolizes polycyclic aromatic hydrocarbons (PAHs) (Ryan et al., 1982) to a variety of products, including activated metabolites that covalently bond to cellular macromolecules and may initiate carcinogenesis (Conney, 1982, and references cited therein).

The carcinogenicities of numerous PAHs have been evaluated and related to their structures (Arcos & Argus, 1974; Wislocki & Lu, 1988; Harvey, 1991). The interaction of different PAHs with P450s and the role of PAH structure in P450-mediated activation remains an important question in the field of PAH-induced carcinogenesis. Regio- and stereospecific relationships between the P450 1A1 heme and PAH binding sites have been inferred from PAH metabolite profiles (Jerina et al., 1982; van Bladeren et al., 1984) and PAH binding studies (Imai, 1982a). However, details of this interaction are unknown because, in contrast to P450cam whose three dimensional structure and mode of substrate and ligand binding has been well-characterized (Raag & Poulos, 1991; Raag et al., 1993, and references cited therein), similar information is unavailable for mammalian P450s.

The catalytic mechanism of P450s involves substrate binding, reduction of ferric heme to the ferrous state, oxygen binding to heme iron followed by its activation, and oxidation of the substrate (White & Coon, 1980; Guengerich & MacDonald, 1990). Since oxygen binding is a crucial step in the catalytic cycle, it is important to elucidate the mechanism of ligand binding to heme. CO has been used as an alternative ligand probe to oxygen since it uniquely yields a photodissociable complex with P450, a property which allows for studying the kinetics of CO binding to P450 by flash photolysis (Koley et al., 1994, and references cited therein). This method entails disruption of the photolabile heme−CO bond by a laser flash and monitoring recombination of CO by the heme absorbance change at 450 nm. The kinetics are sensitive to a variety of factors that influence the rate of CO diffusion through the protein matrix to the heme and provide a valuable probe of P450 conformation and dynamics.

In order to gain further insight into the nature of the PAH−P450 interaction and its impact on P450 conformation and ligand binding, we examined the effects of selected PAHs on the kinetics of CO binding to P450 1A1. However, in contrast to previous studies which evaluated the effects of PAHs on the kinetics of purified P450s (Imai et al., 1982;

* Address correspondence to NIH, Bldg. 37, Room 3E-24, Bethesda, MD 20892. Telephone: 301-496-6365; FAX: 301-496-8419.

[‡] Laboratory of Molecular Carcinogenesis.

[§] Biomedical Instrumentation and Engineering Program.

[⊗] Abstract published in *Advance ACS Abstracts,* January 15, 1995.

[1] Abbreviations: P450, cytochrome P450; MC, 3-methylcholanthrene; MC-microsomes, microsomes from MC-treated rats; PAH, polycyclic aromatic hydrocarbon.

Shimizu et al., 1991), we utilized rat liver microsomes which more closely approximate the natural environment of the P450 in the endoplasmic reticulum. A recently developed kinetic difference method (Koley et al., 1994) was applied to distinguish the kinetics of P450 1A1 from other microsomal P450s. This approach revealed that various PAHs as well as two structurally related flavones differentially modulate the rate of ligand binding and thus alter protein-assisted positioning of substrate, ligand, and heme in the P450 active site. Binding of the ligand oxygen, which is essential for P450-mediated activation of PAHs, may thus also be regulated in a PAH-dependent manner.

MATERIALS AND METHODS

Rat Liver Microsomes. Male Sprague–Dawley rats (8–9 weeks old) were injected intraperitoneally daily with 3-methylcholanthrene (MC) (40 mg/kg of body weight for 3 days) to induce P450 1A1. Liver microsomes were prepared by differential centrifugation and were suspended in 0.25 M sucrose and stored at −80 °C. The microsomal P450 content was spectrally determined by the CO difference method (Omura & Sato, 1964), and the protein concentration was determined by the BCA protein assay (Pierce) using bovine serum albumin as a standard.

Flash Photolysis. Reactions were carried out using 0.39 mg/mL microsomes and 20 μM CO, at 23 °C in 0.1 M sodium phosphate (pH 7.5), 20% glycerol (w/v). When present, PAHs and flavones were added (from a 10 mM stock solution in DMSO) to yield a final concentration of 10 μM, and the mixture was incubated for 20 min before adding CO. Further details were previously described (Koley et al., 1994). The instrumentation for photodissociation of the P450–CO complex and monitoring of reassociation kinetics at 450 nm was previously described (Markowitz et al., 1992).

Data Analysis. Since microsomes contain a multiplicity of P450s, classical multiexponential analysis of CO binding kinetics yields parameters for mixtures of kinetically unresolvable P450s rather than individual P450s. To overcome this problem, we developed a kinetic difference method (Koley et al., 1994) in which the influence of a substrate or other effector for a specific P450 is used to define the kinetic behavior of that P450 in microsomes. Using this approach, kinetic parameters for individual P450s can thus be obtained by least-squares fitting of the data to

$$\Delta A_t' - \Delta A_t = \sum a_i' e^{(-k_i' t)} - \sum a_i e^{(-k_i t)} \qquad (1)$$

where $\Delta A_t'$ and ΔA_t are the absorbance changes observed at time t for the reactions in the presence and absence of substrate; a_i' and a_i are the absorbance changes, and k_i' and k_i are the pseudo-first-order rate constants for the effector-specific P450s in the presence and absence of substrate, respectively. Data were processed and analyzed with RS/1 software (BBN Software Products, Cambridge, MA).

Accessible surface areas of PAHs were determined using Quanta 3.0 software (Molecular Simulations, Waltham, MA) on a Silicon Graphics 4D/70G workstation. Structures were energy minimized with 100 steps of the steepest descents algorithm, and areas were calculated using a 3.0-Å probe.

RESULTS AND DISCUSSION

We examined the effect of representative PAHs of different sizes and shapes (Figure 1) on the kinetics of CO

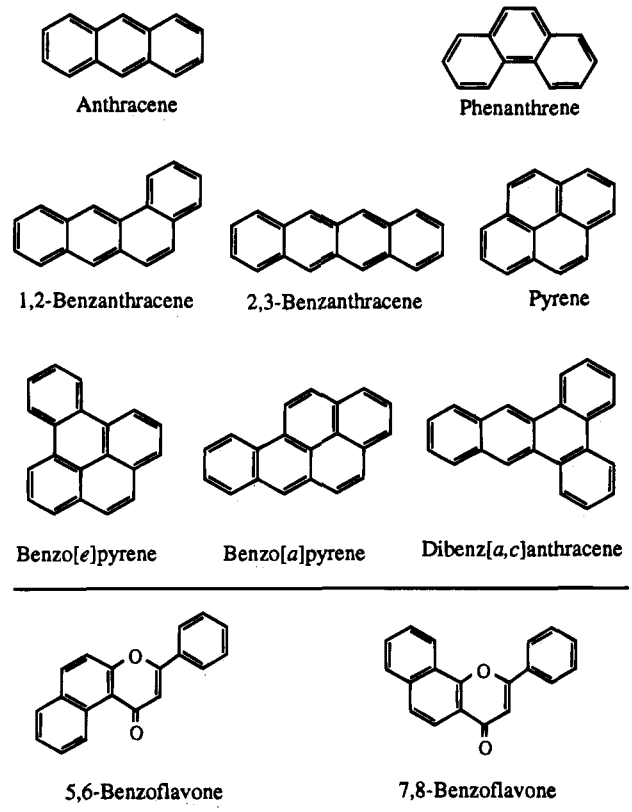

FIGURE 1: Polycyclic aromatic hydrocarbons and flavones evaluated for their effect on the CO binding kinetics of MC-microsomes.

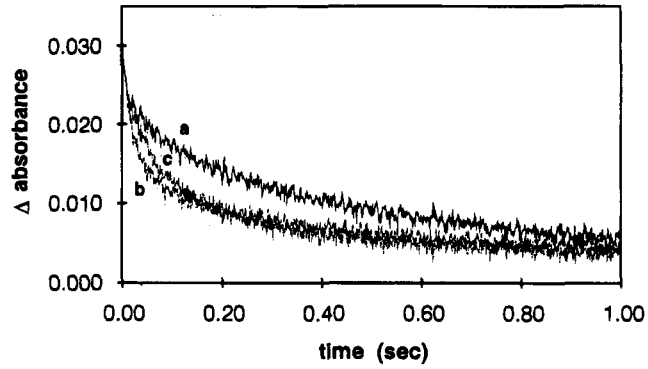

FIGURE 2: Effect of phenanthrene and pyrene on binding of CO to P450s in MC-microsomes. Progress curves (a) in the absence and presence of (b) phenanthrene and (c) pyrene, respectively. The CO concentration was 20 μM; PAH concentration was 10 μM; microsomal concentration was 0.39 mg/mL in 0.1 M sodium phosphate buffer (pH 7.5) containing 20% (w/v) glycerol; temperature, 23 °C.

binding to MC-microsomes. A typical CO binding curve is shown in Figure 2 along with curves obtained in the presence of phenanthrene or pyrene. While both PAHs accelerated CO binding, examination of the early part of the reaction curve (up to ≈0.1 s) clearly shows that phenanthrene was more effective than pyrene. The remaining PAHs likewise accelerated CO binding and yielded distinct reaction profiles (data not shown). Interpretation of CO binding data for microsomes is not straightforward since the contribution of multiple P450s to the overall reaction complicates extraction of kinetic information for a particular PAH-specific P450. We therefore applied a recently developed kinetic difference method (Koley et al., 1994) to our data. This approach evaluates the difference between the kinetic profiles obtained in the presence and absence of the PAH and thus effectively

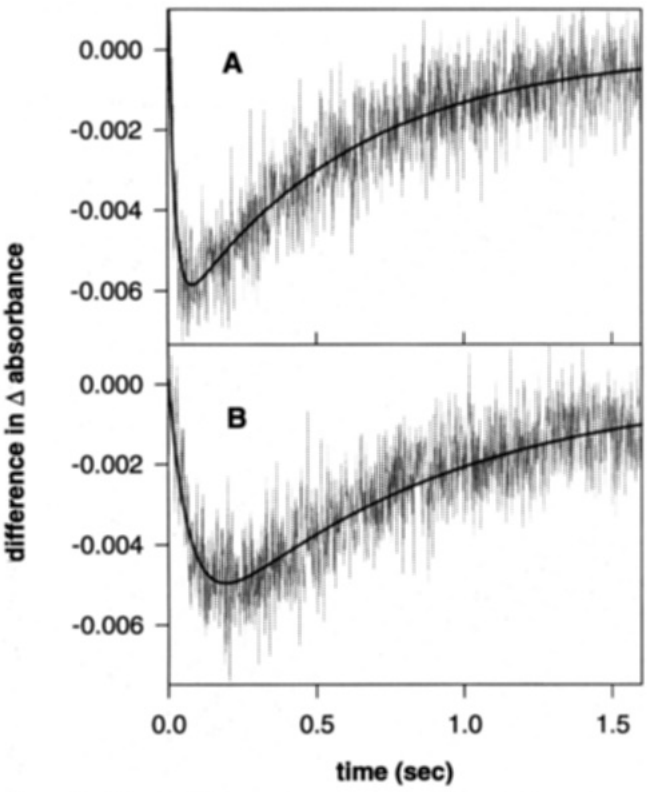

FIGURE 3: Kinetic difference curves for the effect of phenanthrene and pyrene on CO binding to MC-microsomes. (A) Difference between traces b and a in Figure 2 for phenanthrene. (B) Difference between traces c and a in Figure 2 for pyrene. The solid lines represent the best fits according to eq 1.

Table 1: Kinetic Parameters for CO Recombination to P450 1A1 in the Presence and Absence of Different Polycyclic Aromatic Hydrocarbons[a]

PAH	a_1'	k_1' (s^{-1})	a_1	k_1 (s^{-1})
anthracene	0.0064 ± 0.0010	63.8 ± 5.0	0.0053 ± 0.0005	1.3 ± 0.1
phenanthrene	0.0081 ± 0.0005	52.3 ± 4.8	0.0071 ± 0.0003	1.4 ± 0.2
1,2-benzanthracene	0.0104 ± 0.0011	21.5 ± 2.6	0.0078 ± 0.0007	1.4 ± 0.2
2,3-benzanthracene	0.0049 ± 0.0007	11.1 ± 2.5	0.0071 ± 0.0004	1.3 ± 0.1
pyrene	0.0068 ± 0.0006	14.7 ± 1.0	0.0069 ± 0.0002	1.2 ± 0.1
benzo[*e*]pyrene	0.0015 ± 0.0004	25.6 ± 5.8	0.0102 ± 0.0004	1.6 ± 0.2
benzo[*a*]pyrene	0.0086 ± 0.0015	12.7 ± 2.7	0.0100 ± 0.0016	1.5 ± 0.3
dibenz[*a*,*c*]-anthracene	0.0069 ± 0.0028	4.8 ± 1.5	0.0132 ± 0.0061	1.6 ± 0.5

[a] Parameters were determined according to eq 1. k_1 and k_1' are the pseudo-first-order rate constants, and a_1 and a_1' are the corresponding absorbances in the absence and presence of substrate, respectively. Means and standard deviations are derived from at least four experiments.

cancels out the contributions from P450s that do not bind the PAH. Figure 3 shows the resultant difference curves for the data in Figure 2 along with the least squares curve fit to eq 1. This procedure yielded k_1 and k_1', which represent the CO binding rate constants for a single PAH-specific P450 in the absence and presence of the PAH, respectively. The kinetic parameters are presented in Table 1. For the data presented in Figure 2, this analysis revealed that phenanthrene accelerated the rate of CO binding to this P450 by 37-fold (from 1.4 to 52.3 s^{-1}) while pyrene accelerated the rate 12-fold (from 1.2 to 14.7 s^{-1}). Analyses of experiments with the remaining PAHs in Figure 1 likewise revealed that these accelerated the rate of a single P450 to varying degrees (Table 1). Furthermore, the similar k_1 values (1.2−1.6 s^{-1}) suggest a common target P450 for these PAHs.

To ascertain whether the PAH-sensitive P450 was the MC-inducible and PAH-metabolizing P450 1A1, we evaluated the effect of PAHs on CO binding by control microsomes from untreated rats, which lack 1A1 (Guengerich et al., 1982; Luster et al., 1983). We found that none of the PAHs altered the kinetics (data not shown), which indicates that the PAH-accelerated rate observed in MC-microsomes derives from P450 1A1. Although the P450 1A2 form is also MC-inducible, it is unlikely to correspond to the PAH-sensitive P450 for several reasons: (1) Comparing the absorbance changes for the PAH-sensitive P450 (a_1, Table 1) to the absorbance change for total microsomal P450 ($\Delta A_0 = 0.033$, Figure 1) indicates that PAHs interact with a P450 that constitutes 16−40% of total P450. The changes thus cannot derive from P450 1A2, a minor form comprising only 12% of the total P450 in MC-microsomes, whereas P450 1A1 can account for the observed changes as it constitutes 56% of the total P450 in these microsomes (Dannan et al., 1983). (2) PAHs such as benzo[*a*]pyrene are poorly metabolized by P450 1A2 but are efficiently metabolized by P450 1A1 (Ryan et al., 1982). (3) Phenanthrene, dibenz[*a*,*c*]anthracene and 7,8-benzoflavone decreased, and anthracene had no effect on the kinetics of CO binding to purified P450 1A2 (Shimizu et al., 1991); in contrast, we found that these PAHs (including the flavone, whose data are presented later) accelerated CO binding to the PAH-sensitive P450. These considerations thus strongly implicate P450 1A1 rather than 1A2 as the PAH-sensitive P450 responsible for the changes in CO binding kinetics. One must also consider, however, that although P450 1A2 is not solely responsible for the observed changes in kinetics, it may partially contribute to an overall change largely derived from P450 1A1. This is unlikely since the kinetic difference analysis detected a single kinetically distinguishable PAH-sensitive P450, and any contribution from P450 1A2 would thus be undetectably small.

The results in Table 1 show that PAHs of varying molecular size and shape differentially alter the rate of CO binding to P450 1A1. The wide range in rates of PAH-bound P450 1A1 is exemplified by the smallest and largest PAHs, phenanthrene and dibenz[*a*,*c*]anthracene, which yielded k_1' values of 52.3 and 4.8 s^{-1}, respectively. The effects of PAHs are firstly and most simply classified according to size as gauged by the number of aromatic rings: the tricyclic PAHs, anthracene and phenanthrene, enhanced the rate to the greatest extent (by 49- and 37-fold, respectively), while the larger tetracyclic and pentacyclic PAHs enhanced the rates 3−16-fold. As a basis for further interpreting these data in terms of PAH size, the accessible surface areas of the PAHs were calculated. This parameter reflects both the potential interaction surface between a PAH and its complementary P450 binding region as well as the degree to which the PAH sterically hinders CO binding to heme. A plot of the surface areas verus the log of the respective rate constants (Figure 4) reveals that these are correlated ($r = -0.83$). However, molecular shape also influences the rate: although only relatively slight differences were observed in the presence of the two tricyclic PAHs (63.8 and 52.3 s^{-1}), appreciable differences (11.1−21.5 s^{-1}) were observed among the tetracyclic compounds, and the pentacyclic PAHs exhibited the greatest variability (4.8−25.6 s^{-1}) (Table 1).

We interpret these findings in terms of a dual mechanism: (1) P450 substrates can modify the P450 conformation/

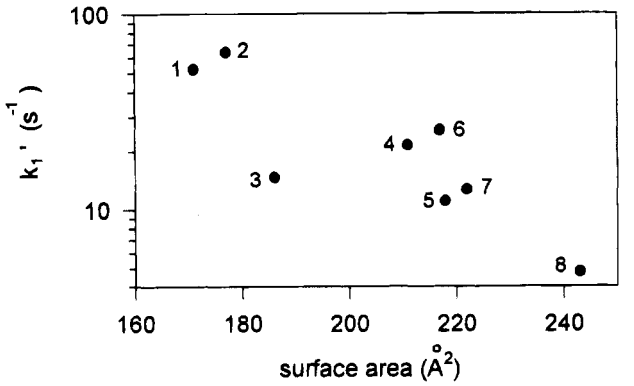

FIGURE 4: Accessible surface areas of the polycyclic aromatic hydrocarbons and corresponding pseudo-first-order rate constants for CO binding to MC-microsomes: (1) phenanthrene, (2) anthracene, (3) pyrene, (4) 1,2-benzanthracene, (5) 2,3-benzanthracene, (6) benzo[*e*]pyrene, (7) benzo[*a*]pyrene, and (8) dibenz[*a*,*c*]anthracene.

dynamics of the ligand access channel to either inhibit or enhance CO binding, depending on the substrate or P450 form (Koley et al., 1994); and (2) a substrate may sterically hinder diffusion of CO to the heme iron and reduce the binding rate (Peterson & Griffin, 1972), with larger substrates being more effective inhibitors of CO binding. Our observed rates with PAHs thus are a combination of two opposing factors. The first mechanism is always operative and widens the CO access channel since all PAHs accelerated CO binding to P450 1A1. The second factor is evident since the rate enhancement is lessened with increasing PAH size.

Table 1 also shows that PAHs alter the relative magnitudes of the absorbance parameters a_1' and a_1, which reflect the amount of photodissociated CO that diffuses into the solvent in the presence and absence of PAH, respectively. The largest changes were observed with 2,3-benzanthracene, benzo[*e*]pyrene, and dibenz[*a*,*c*]anthracene whose a_1' values were considerably smaller than the corresponding a_1 values for the respective PAH-free P450. While 1,2-benzanthracene slightly increased the absorbance ($a_1' = 0.0104$ and $a_1 = 0.0078$), the remaining PAHs had little effect. The differences between a_1 and a_1' do not derive from different absorbances of the heme−CO complex in the presence and absence of the PAHs, since these PAHs did not appreciably (<5%) change the static CO difference spectra of MC-microsomes (data not shown). The differences between a_1 and a_1' thus show that PAHs alter the heme−CO photodissociation efficiency. Studies with P450cam show that the substrate cam increases the photodissociation efficiency (Shimada et al., 1979) owing to altered π-bonding and/or bending of the heme iron−CO bond (Iizuka et al., 1979; Raag & Poulos, 1989). By this mechanism, substrates would only be expected to increase the efficiency. The increased absorbance with 1,2-benzanthracene is consistent with this interpretation. However, since three PAHs significantly decreased the absorbance, we conclude that the photodissociation efficiency does not simply correlate with the absorbance. Another factor is presumably operative that decreases the absorbance, such as sterically hindering expulsion of photodissociated CO from the vicinity of the heme by the substrate, resulting in rapid geminate recombination of iron and CO. Such a mechanism has indeed been demonstrated for myoglobin (Carver et al., 1990, 1991, and references cited therein), where mutagenesis of certain amino acids in the CO access channel inhibits CO expulsion. These PAHs thus may similarly hinder expulsion of photodissociated CO from the heme pocket of P450 1A1.

The effect of various PAHs on CO binding kinetics was previously reported both by Imai et al. (1982) for a P450 from liver microsomes of phenobarbital-treated rabbits and by Shimizu et al. (1991) for rat P450 1A2. The findings of the former study agreed with ours in that the smaller PAHs (phenanthrene and anthracene) greatly accelerated binding; however, larger PAHs such as dibenz[*a*,*c*]anthracene decreased the rate, in contrast to our finding that this PAH also increased the rate, albeit to a lesser degree than the smaller PAHs. As mentioned previously, our results significantly differed from those of Shimizu et al. (1991). These divergent observations may most simply be attributed to the difference in P450 forms: rabbit P450 2B4 (Imai et al., 1982) and rat P450 1A2 (Shimizu et al., 1991) versus P450 1A1 in the present work.

P450 1A1 substrates such as PAHs are rigid planar molecules with a large area/depth ratio and small depth (Lewis et al., 1986). A model of the substrate binding site has been deduced through studies of benzo[*a*]pyrene metabolism (Jerina et al., 1982) and has been used to explain the metabolism of PAHs and related compounds by P450 1A1 (Vyas et al., 1983; van Bladeren et al., 1984). Although this model outlines a minimal binding site, it did not adequately explain the metabolism of several PAHs that do not fit into the substrate binding site (Yang et al., 1985). Our results suggest caution in utilizing a static model for the substrate binding site because PAHs differentially modify P450 conformation/dynamics. This suggests that the substrate binding site is not rigid but is shaped in part by the nature of the substrate. This interpretation is consistent with the view that an induced fit may be preferred to the classical lock and key model of binding of small molecules to proteins (Jorgensen, 1991).

In addition to PAHs, we also evaluated the effect of two related compounds, 5,6-benzoflavone and 7,8-benzoflavone, on the CO binding kinetics of MC-microsomes. Although similar to the tetracyclic PAHs in the number of aromatic rings, these flavones include a rotatable phenyl group and a heterocyclic ring (Figure 1). These compounds have interesting properties as they inhibit benzo[*a*]pyrene hydroxylation by MC-microsomes but enhance this activity in microsomes from untreated rats (Wiebel et al., 1971; Nesnow, 1979; Friedman et al., 1985). These observations are consistent with studies that showed that both flavones interact with the P450 1A1 in MC-microsomes (Vyas et al., 1983; Andries et al., 1990) as well as with several constitutive P450s (Wiebel, 1980; Huang et al., 1981; Vyas et al., 1983; Schwab et al., 1988). In addition, like PAHs, these flavones are metabolized by P450 1A1 (Vyas et al., 1983; Andries et al., 1990).

The effects of 5,6-benzoflavone on CO binding to MC- and control microsomes are presented in Figure 5. These firstly show that in control microsomes, the flavone decreased the rate. Kinetic difference analysis revealed that 5,6-benzoflavone decreased the rate of CO binding to a constitutive P450 in these microsomes by 15-fold (from 229.3 to 15.7 s^{-1}) (Table 2). The effect of this flavone on MC-microsomes was more complex, as two phases were evident: the rate of CO binding was initially decreased but increased at later times. Kinetic difference analysis revealed

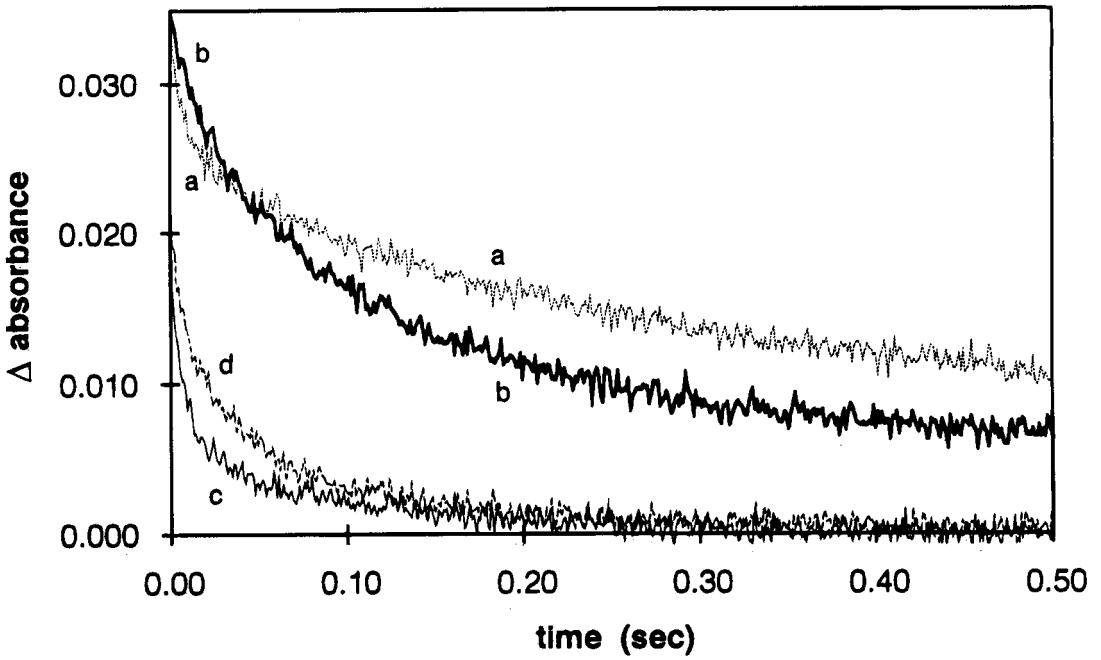

FIGURE 5: Effect of 5,6-benzoflavone on the binding of CO to P450s in MC- and control microsomes. Progress curves for MC-microsomes: (a) in the absence and (b) presence of 5,6-benzoflavone. Progress curves for control microsomes: (c) in the absence and (d) presence of 5,6-benzoflavone. 5,6-Benzoflavone concentration was 10 μM; other conditions and concentrations are the same as in Figure 2. Although data were collected for the complete reaction (1.6 s), only the initial 0.5 s is shown in order to clearly show the early part of the reaction.

Table 2: Effect of Flavones on CO Recombination to P450[a]

	a_1'	k_1' (s^{-1})	a_1	k_1 (s^{-1})	a_2'	k_2' (s^{-1})	a_2	k_2 (s^{-1})
MC-microsomes + 5,6-benzoflavone	0.0032 (0.0009)[b]	17.2 (8.3)	0.0033 (0.0003)	252.0 (40.5)	0.0088 (0.0018)	5.6 (1.4)	0.0084 (0.0018)	1.6 (0.3)
control microsomes + 5,6-benzoflavone	0.0039 (0.0002)	15.7 (1.8)	0.0036 (0.0009)	229.3 (31.6)				
MC-microsomes + 7,8-benzoflavone	0.0040 (0.0002)	29.0 (1.8)	0.0103 (0.0007)	1.6 (0.2)				
control microsomes + 7,8-benzoflavone	0.0030 (0.0002)	14.8 (5.3)	0.0024 (0.0005)	307.7 (27.8)				

[a] Parameters were determined according to eq 1. k_1 and k_2 are the pseudo-first-order rate constants in the absence of benzoflavone while k_1' and k_2' are the rate constants in the presence of benzoflavone. [b] Standard deviations (derived from at least three experiments) are given in parentheses.

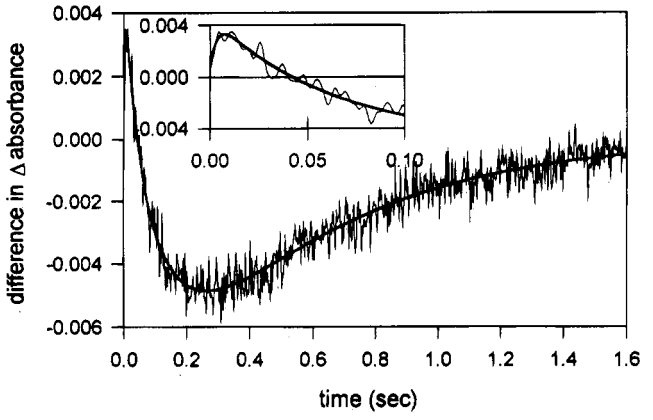

FIGURE 6: Kinetic difference curve for the effect of 5,6-benzoflavone on CO binding kinetics to MC-microsomes. The curve represents the difference between traces b and a in Figure 5. The solid line corresponds to the best fit according to eq 1. The early part of the difference curve is expanded in the inset.

that two P450s were affected (Figure 6) and yielded parameters for each P450 in the absence and presence of the flavone. The set of parameters with the higher values (k_1 and k_1') corresponds to those of the constitutive P450 in control microsomes and suggests it is also present in MC-microsomes. Interaction of the flavone with this P450 is thus responsible for the decreased rate in the early stage of the reaction with MC-microsomes. On the other hand, the rate of the latter phase of MC-microsomes (corresponding to k_2 and k_2') was enhanced 3.5-fold in the presence of 5,6-benzoflavone (from 1.6 to 5.6 s^{-1}). In addition to providing information on the effect of flavone on both P450 1A1 and a constitutive P450, the results also allow comparison of the rates for two P450s within a single microsomal sample. The great rate difference between the flavone-free P450s (1.6 vs 252 s^{-1}) indicates major differences between the conformation of these P450s as reflected in ligand access to the heme. The opposing effects of flavone on the CO binding rate, an enhancement with P450 1A1 and reduction with the constitutive P450, further points to major P450-specific differences in responsiveness to a given substrate molecule. Thus while examination of the raw data (Figure 5) readily shows that control and MC-microsomes vary in their overall rate of CO binding, our analysis more precisely defines this difference in terms of their constituent P450s.

In contrast to 5,6-benzoflavone, 7,8-benzoflavone uniformly accelerated the rate and additionally reduced the absorbance amplitude of CO binding to MC-microsomes (data not shown). Difference analysis revealed a rate enhancement of 18-fold for a single P450 (Table 2). The increased rate and similarity of the value of k_1 (1.6 s^{-1}) to that obtained for the PAHs (Table 1) suggest that the difference also derives from P450 1A1. However, although this flavone inhibits P450 1A1 (Wiebel, 1980), it is known

to also interact with constitutive P450s, including P450 3A (Schwab et al., 1988). We thus assessed its effect on control microsomes from untreated rats and found it decreased the CO binding rate of a single P450 by 21-fold (Table 2). Since the results with MC-microsomes showed that the sole effect of this flavone was rate enhancement for a single P450, the 7,8-benzoflavone-sensitive P450 in control microsomes is absent or undetectable in MC-microsomes. The different effects of 5,6- and 7,8-benzoflavone on P450 1A1 (rate enhancements of 3.5- and 18-fold, respectively) and their different selectivities for constitutive P450s thus illustrate the sensitivity of ligand binding to substrate structure.

The MC-inducible P450 1A1 form efficiently metabolizes PAHs relative to other P450s (Ryan et al., 1982) and is primarily responsible for PAH activation (Gozukara et al., 1982). However details of the PAH interaction with this hemeprotein remain unclear since the three-dimensional structure of a mammalian P450 has not been determined. The effect of a PAH substrate on the CO binding kinetics offers a unique approach to probe the interaction of substrate with P450 heme and protein, since it is sensitive to both structure and dynamics. The results indicate that PAHs exert a dual effect both by altering protein conformation/dynamics to widen the CO access channel to enhance binding and by sterically hindering binding. The divergent effects of two structurally related flavones on P450 1A1 and a constitutive P450 further demonstrate the sensitivity of P450 ligand binding to substrate structure.

REFERENCES

Andries, M. J., Lucier, G. W., Goldstein, J., & Thompson, C. L. (1990) *Mol. Pharmacol. 37*, 990−995.

Arcos, J. C., & Argus, M. F. (1974) in *Chemical Induction of Cancer, Structural Bases and Biological Mechanisms* (Wolf, G., Ed.) Vol. IIA, pp 15−65, Academic Press, New York.

Carver, T. E., Rohlfs, R. J., Olson, J. S., Gibson, Q. H., Blackmore, R. S., Springer, B. A., & Sligar, S. G. (1990) *J. Biol. Chem. 265*, 20007−20020.

Carver, T. E., Olson, J. S., Smerdon, S. J., Krzywda, S., Wilkinson, A. J., Gibson, Q. H., Blackmore, R. S., Dezz Ropp, J., & Sligar, S. G. (1991) *Biochemistry 30*, 4697−4705.

Dannan, G. A., Guengerich, F. P., Kaminsky, L. S., & Aust, S. D. (1983) *J. Biol. Chem. 258*, 1282−1288.

Friedman, F. K., Wiebel, F. J., & Gelboin, H. V. (1985) *Pharmacology 31*, 194−202.

Gozukara, E. M., Guengerich, F. P., Miller, H., & Gelboin, H. V. (1982) *Carcinogenesis 3*, 129−133.

Guengerich, F. P., & MacDonald T. L. (1990) *FASEB J. 4*, 2453−2459.

Guengerich, F. P., Ghazi, A. D., Wright, S. T., Martin, M. V., & Kaminsky, L. S. (1982) *Biochemistry 21*, 6019−6030.

Harvey, R. G. (1991) in *Polycyclic Aromatic Hydrocarbons: Chemistry and Carcinogenicity*, pp 26−95, Cambridge University Press, Cambridge.

Huang, M. T., Johnson, E. F., Muller-Eberhard, A., Koop, D. R., Coon, M. J., & Conney, A. H. (1981) *J. Biol. Chem. 256*, 10897−10901.

Iizuka, T., Shimada, H., Ueno, R., & Ishimura, Y. (1979) in *Cytochrome Oxidase* (Chance, B., King, T. E., Okunuki, K., & Orii, Y., Eds.) pp 9−20, Elsevier, Amsterdam.

Imai, Y. (1982) *J. Biochem. 92*, 77−88.

Imai, Y., Iizuka, T., & Ishimura, Y. (1982) *J. Biochem. 92*, 67−75.

Jerina, D. M., Michaud, D. P., Feldmann, R. S., Armstrong, R. N., Vyas, K. P., Thakker, D. R., Yagi, H., Ryan, D. E., Thomas, P. E., & Levin, W. (1982) in *Microsomes, Drug Oxidations and Drug Toxicity* (Sato, R., & Kato, R., Eds.) pp 195−201, Japan Scientific Societies Press, Tokyo.

Jorgensen, W. L. (1991) *Science 254*, 954−955.

Koley, A. P., Robinson, R. C., Markowitz, A., & Friedman F. K. (1994) *Biochemistry 33*, 2484−2489.

Lewis, D. F. V., Ioannides, C., & Parke, D. V. (1986) *Biochem. Pharmacol. 35*, 2179−2185.

Lu, A. Y. H., & West, S. B. (1980) *Pharmacol. Rev. 31*, 277−295.

Luster, M. I., Lawson, L. D., Linko, P., & Goldstein, J. A. (1983) *Mol. Pharmacol. 23*, 252−257.

Markowitz, A., Robinson, R. C., Omata, Y., & Friedman, F. K. (1992) *Anal. Instrum. 20*, 213−221.

Nesnow, S. A. (1979) *J. Med. Chem. 22*, 1244−1247.

Omura, T., & Sato, R. (1964) *J. Biol. Chem. 239*, 2370−2385.

Ortiz de Montellano, P. R., Ed. (1986) *Cytochrome P-450: Structure, Mechanism and Biochemistry*, Plenum Press, New York.

Peterson, J. A., & Griffin, B. W. (1972) *Arch. Biochem. Biophys. 151*, 427−433.

Raag, R., & Poulos, T. L. (1989) *Biochemistry 28*, 7586−7592.

Raag, R., & Poulos, T. L. (1991) *Biochemistry 30*, 2674−2684.

Raag, R., Li, H., Jones, B. C., & Poulos, T. L. (1993) *Biochemistry 32*, 4571−4578.

Ryan, D. E., & Levin, W. (1990) *Pharmacol. Ther. 45*, 153−239.

Ryan, D., Thomas, P. E., Reik, L. M., & Levin, W. (1982) *Xenobiotica 12*, 727−744.

Shimada, H., Iizuka, T., Ueno, R., & Ishimura, Y. (1979) *FEBS Lett. 98*, 290−294.

Shimizu, T., Ito, O., Hatano, M., & Fujii-Kuriyama, Y. (1991) *Biochemistry 30*, 4659−4662.

Thomas, P. E., Reik, L. M., Ryan, D. E., & Levin, W. (1981) *J. Biol. Chem. 256*, 1044−1052.

van Bladeren, P. J., Vyas, K. P., Sayer, J. M., Ryan, D. E., Thomas, P. E., Levin, W., & Jerina, D. M. (1984) *J. Biol. Chem. 259*, 8966−8973.

Vyas, K. P., Shibata, T., Highet, R. J., Yeh, H. J., Thomas, P. E., Ryan, D. E., Levin, W., & Jerina, D. M. (1983) *J. Biol. Chem. 258*, 5649−5659.

White, R. E., & Coon, M. J. (1980) *Annu. Rev. Biochem. 49*, 315−356.

Yang, S. K., Mushtaq, M., & Chiu, Pei-Lu (1985) in *Polycyclic Hydrocarbons and Carcinogenesis* (Harvey R. G., Ed.) ACS Symposium Series 283, pp 19−34, Washington, DC, American Chemical Society.

Wiebel, F. J. (1980) in *Carcinogenesis: Modifiers of Chemical Carcinogenesis* (Slaga, T. J., Ed.) Vol. 5, pp 57−84, Raven Press, New York.

Wiebel, F. J., Leutz, J. C., Diamond, L., & Gelboin, H. V. (1971) *Arch. Biochem. Biophys. 144*, 78−86.

Wislocki, P. G., & Lu, Y. H. (1988) in *Polycyclic Aromatic Hydrocarbon Carcinogenesis: Structure-Activity Relationships* (Yang, S. K., & Silverman, B. D., Eds.) Vol I, pp 1−30, CRC Press, Boca Raton, FL.

BI9420323