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A PARTITION MODEL FOR HEPATIC CYTOCHROME P-450-HYDROCARBON COMPLEX FORMATION

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Many substrates for hepatic oxidative metabolism bind to cytochrome P-450, producing small but significant changes in its absorbance spectrum (1,2). Type I substances, typified by hexobarbital, produce an increase in absorbancy at about 385 nm and a decrease at about 420 nm. A number of aromatic compounds are oxidatively metabolized by liver microsomes. Among the simple aromatic hydrocarbons, benzene, naphthalene and ethylbenzene have been found to induce type I spectral changes in hepatic microsomes (3,4,5). Hydrocarbon binding to other enzymes (6,7) has been shown to obey a "partition" law, the hydrocarbon behaving as if "extracted" from the aqueous medium, with enzyme representing the nonaqueous phase. If cytochrome P-450 possesses a similar hydrophobic binding site, any substance with hydrocarbon character should bind effectively. Hydrophobic interactions between fatty acids and renal cytochrome P-450 have been postulated (8) but carboxyl groups were considered essential. To determine the significance of hydrophobicity in substrate binding to cytochrome P-450, we have now examined hydrocarbon-induced spectral changes in hepatic microsomes.

Male Sprague-Dawley rats, 60-70 days old were decapitated between 8 and 9 a.m. and liver microsomes prepared as described by Omura and Sato (9). Hydrocarbon-induced difference spectra (ΔOD 385-420) were obtained using a

Cary 17 recording spectrophotometer at room temperature. Microsomal suspensions contained 3-4 mg protein/ml. Spectral association constants were calculated by the method of Schenkman et al. (10).

Benzene, ethylbenzene, toluene, xylene, indene and naphthalene produced typical type I difference spectra upon addition to hepatic microsomal suspensions. The relative maximum changes in absorbance (ΔOD max $_{385-420}$) produced by each is indicated in figure 1A. Studies with yeast alcohol dehydrogenase and α-chymotrypsin (6,7) demonstrated that the dependence of free energy of complex formation upon molecular size for this series of hydrocarbons was the

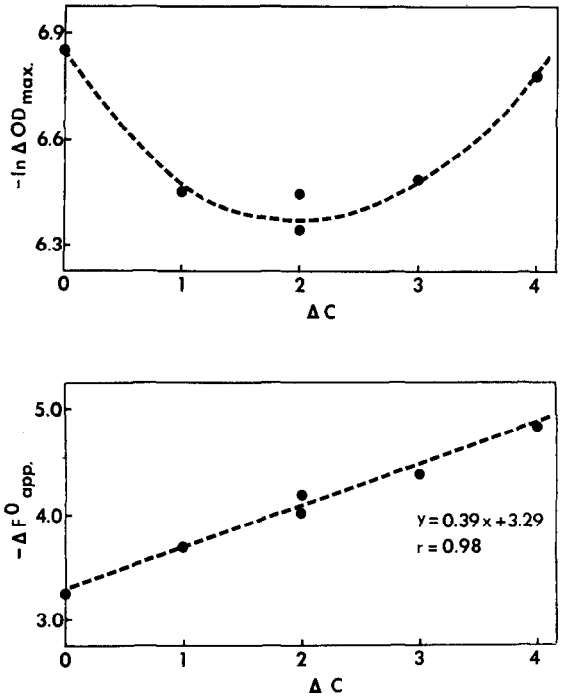

Figure 1A (Top) ΔOD max vs ΔC, the increment of carbon atoms added. From left to right the points represent benzene, toluene, ethyl benzene and p-xylene, indene and naphthalene.

Figure 1B (Bottom) Apparent free energy of complex formation vs ΔC for the same series of hydrocarbons. Each point is the mean of three values.

same as predicted if the enzyme acted as a second phase and the hydrocarbon were completely transferred from aqueous phase to enzyme. Thermodynamic considerations predicted and experiments confirmed that a plot of ΔF^{o}_{APP}, apparent free energy change of complex formation, vs aromatic hydrocarbon molecular surface area produced a straight line with a slope of approximately 0.12 (the equivalent of approximately 0.75 kcal per carbon atom added). One may use either ΔC or surface area since good linearity is obtained with either. Figure 1B indicates that hydrocarbon interactions with hepatic cytochrome P-450 follow a similar law. The slope of 0.39 resulting from the plot of hydrocarbon size vs (spectral) ΔF^{o} is smaller than that expected for the transfer of hydrocarbon from water to enzyme. This infers that the partition may be between a less polar "solvent" (microsome?) and cytochrome P-450. At any rate, figure 1B shows that the relative abilities of the aromatic hydrocarbons to bind to cytochrome P-450 are directly proportional to their tendencies to leave an aqueous solution and enter a hydrocarbon phase.

The partition model would explain the effect of butanol for example, which has been shown to remove substrates from cytochrome P-450 (11) on the basis of a change in the chemical potential of the substrate in the enzyme's environment. There may be a competitive mechanism operating as well, but according to this approach the main effect would be to "leech" the substrate from the enzyme.

Type I spectra were also produced by the non-aromatic compounds cyclohexene, n-hexane and n-pentane (the association constant for each being 970, 830, and 371 respectively). Thus, neither charge transfer complex formation nor a cyclic nor a flat molecule is essential for hydrocarbon-induced spectral changes. The only apparent requirement is hydrophobicity. Since there is no tendency toward curvature in Fig. 1B, it might be expected that binding of larger hydrocarbons such as the cholanthrenes would be very large indeed. Binding of aromatic hydrocarbons is simply dependent upon molecular size. (The association constant for benzene is 230, for naphthalene 3500.) These

results provide evidence that hydrophobic interaction is the greatest single factor in the binding of substrates to hepatic cytochrome P-450. Since hydrophobic interactions represent the sole mechanism for hydrocarbon-induced spectral changes in cytochrome P-450, these compounds will provide useful probes in further studies to evaluate the apparent hydrophobicity of cytochrome P-450 and/or its environment under various experimental conditions.

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