SPECTRAL INTERACTIONS OF A SERIES OF CHLORINATED HYDROCARBONS WITH CYTOCHROME *P*-450 OF LIVER MICROSOMES FROM VARIOUSLY-TREATED RATS

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1. Introduction

The cytochrome *P*-450-linked mono-oxygenase system which resides in the liver microsomes and also in other tissues metabolizes a vast number of foreign as well as endogenous compounds, including drugs, steroids, pesticides etc. [1]. The pre-requisite for the metabolism is supposed to be a binding of substrate to oxidized cytochrome *P*-450 which is often observable in the difference spectrum of the microsomes as a type I spectral change, characterized by a trough at about 420 nm and a peak at about 390 nm [2,3].

Because the cytochrome *P*-450-linked mono-oxygenase system of liver microsomes is very unspecific with respect to metabolizable substrates there have been attempts to find common properties for the heterogeneous group of cytochrome *P*-450 substrates. Gaudette and Brodie [4] were first to point out that there appears to be a direct relationship between the rate of microsomal metabolism and the lipophilic character of drugs. Later, McMahon [5] and Martin and Hansch [6] found rough correlations between lipid solubility and metabolism by the cytochrome *P*-450 system for various groups of compounds. On the other hand, other studies that although lipid solubility may be required for a substance to be metabolized by, and to interact with microsomal cytochrome *P*-450, other properties of the molecule, e.g. stereoselectivity, are of major importance for determining the affinity with which it will interact with the cytochrome [7–9].

In the present study, a series of chlorinated hydrocarbons, methanes, ethanes, ethanes have been studied with respect to spectral interactions with cytochrome *P*-450 of liver microsomes from control, phenobarbital-treated, and 3-methyl-cholanthrene-treated rats. We found that the addition of these compounds resulted in type I spectral interactions. The systematic change in the molecule (the increasing number of chlorine atoms) resulted in a systematic change in the biochemical parameter (the increased magnitude of the type I spectral change and the increased affinity to cytochrome *P*-450). Because the successive addition of chlorine atoms to the hydrocarbon skeleton leads to the increase in hydrophobicity or lipid solubility of the compound [10] we conclude that the important property of the molecule for deciding the affinity to cytochrome *P*-450 is lipid solubility, at least in this series of chlorinated hydrocarbons.

2. Materials and methods

Male rats of Sprague-Dawley strain weighing 250 to 300 g were used. Animals were treated by phenobarbital (500 mg/l in drinking water for 7 days) or 3-methylcholanthrene (20 mg/kg body weight for three days i.p. in sesame oil). Animals were killed 24 hr after stopping the treatment. The liver was immediately removed, washed, and homogenized in 4 vol of 0.1 M K/Na phosphate buffer, pH 7.4, by a glass-teflon homogenizer. After centrifugation of homogenate at 10 000 g for 20 min, the microsomal fraction was obtained by centrifugation at 100 000 g for 60 min. The microsomal pellet was suspended in 0.1 M Tris–HCl, pH 7.45, at a protein concentration of about 20 to 30 mg per ml. Protein concentrations were determined by the Biuret method [11].

For determination of spectral interactions, the microsomal suspension was diluted with Tris–HCl to yield a protein concentration of about 1 to 4 mg/ml. All spectra were measured at room temperature in 1-cm path-length cells in a Shimadzu model MPS-50L recording spectrophotometer. Microsomal suspension was divided into two cuvettes and the baseline was adjusted. Difference spectra were then obtained by addition of μl quantities of the compound in acetone (vol up to 50 μl/3 ml) to one of the cuvettes, while an equal vol of acetone was added to the reference cuvette. Spectra were traced immediately after the addition of the substances. The extent of spectral changes was measured by the difference between the wavelengths of minimum (at 420 nm) and maximum (at 385–390 nm) absorption. Spectral dissociation constants [3] were obtained from double-reciprocal plots of hydrocarbon concentration and spectral change.

The amount of cytochrome *P*-450 in microsomes was measured according to the method of Omura and Sato [12].

3. Results

Fig.1 and 2 shows that all compounds belonging to chlorinated methanes, ethanes, and ethenes except dichloromethane give a type I spectral change with at least some rat liver microsomal preparations. Fig.1 represents an example of spectral changes after the addition of chlorinated ethanes to the microsomal suspension from phenobarbital-treated rat liver and this experiment gave us the first hint of a systematic change of this biochemical parameter and prompted us for further studies.

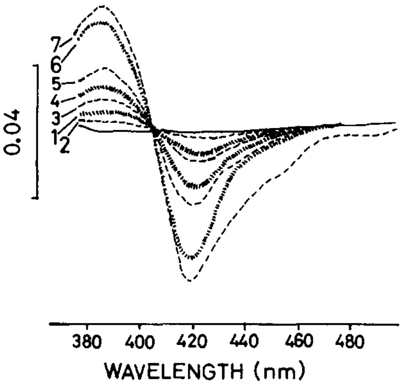

Fig.1. Spectral interactions of 1,1-dichloroethane (1), 1,2-di-chloroethane (2), 1,1,1-trichloroethane (3), 1,1,2-trichloroethane (4), 1,1,2,2-tetrachloroethane (5), pentachloroethane (6), and hexachloroethane (7) with liver microsomes from phenobarbital-treated rats. The final concentrations of the compounds were 1 mM (1–5), 0.5 mM (6), and 0.2 mM (7). The concentration of cytochrome *P*-450 was 2.2 nmol/ml.

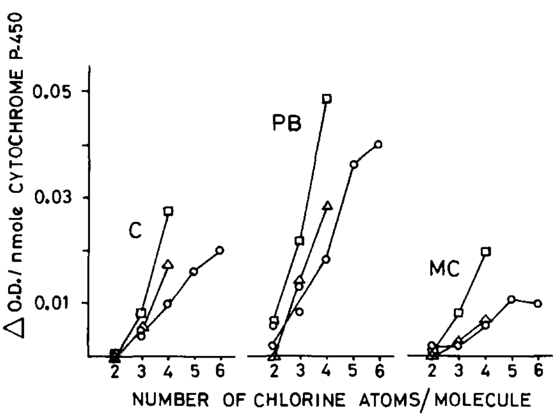

Fig.2. Magnitude of spectral interactions of chlorinated hydrocarbons with liver microsomes from control (C), phenobarbital-treated (PB), and 3-methylcholanthrene-treated (MC) rats. (△ —— △) methane series; (○ —— ○) ethane series; (□ —— □) ethene series.

Fig.2 shows the magnitude of spectral interactions with liver microsomes from control, phenobarbital-treated, and 3-methylcholanthrene-treated rats. Two facts are evident in this figure: the magnitude of the spectral change increases when more chlorine atoms are added to the carbon skeleton, and the magnitudes of type I spectral changes, when calculated on the basis of unit concentration of cytochrome *P*-450, differed much in preparations from variously-treated rats. The 'specific spectral changes' were the largest in the phenobarbital-treated group and the smallest in the 3-methylcholanthrene group.

Fig.3 shows the double-reciprocal plots for the interaction of chlorinated ethanes with liver microsomes from phenobarbital-treated rats. It is clear that the more chlorine atoms in the molecule, the smaller the spectral dissociation constant (K_S), indicating higher affinity to cytochrome P-450. However, with hexachloroethane, pentachloroethane, and

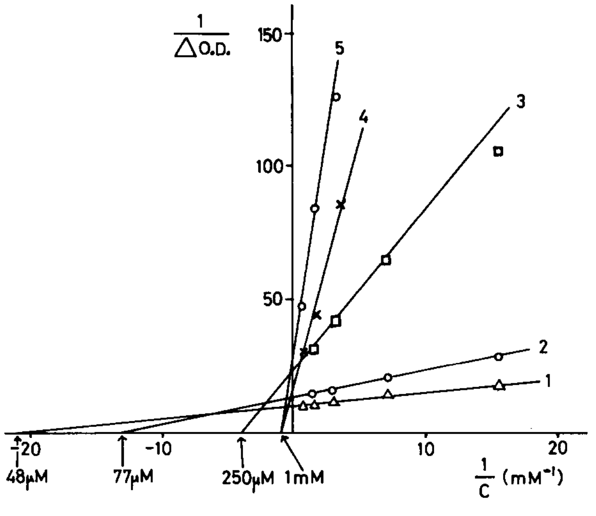

Fig.3. Double-reciprocal plots of the interaction of hexachloroethane (1), pentachloroethane (2), 1,1,2,2-tetrachloroethane (3), 1,1,2-trichloroethane (4), and 1,1,1-trichloroethane (5) with the liver microsomal cytochrome P-450 from phenobarbital-treated rats.

tetrachloroethene, plots are not linear, but apparently two-phasic (fig.4). This could be interpreted as if in phenobarbital-treated liver microsomes there exist two binding sites for chlorinated hydrocarbons with differing affinities ('low' and 'high' affinity binding sites). In table 1 K_S values for the interaction of some chlorinated hydrocarbons with microsomal cytochrome

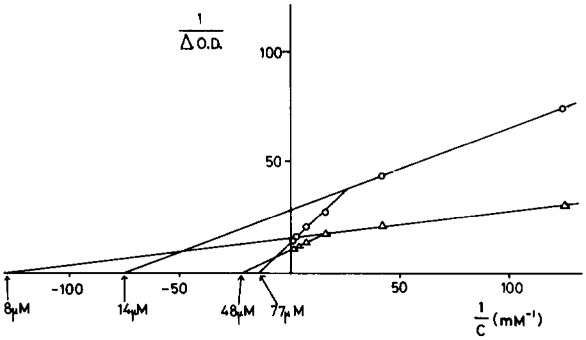

Fig.4. Double-reciprocal plots of the interaction of pentachloroethane (○ —— ○) and hexachloroethane (△ —— △) with liver microsomal cytochrome P-450 from phenobarbital-treated rats.

P-450 are shown. For pentachloroethane, hexachloroethane, and tetrachloroethene K_S values in control and 3-methylcholanthrene-treated microsomes were roughly similar, but in phenobarbital-treated microsomes they were much smaller.

4. Discussion

Since the study of Gaudette and Brodie [4] the accepted thought has been that drug oxidation depends on the lipid solubility of the compound, thus implicating that substrate binding sites of drug-oxidizing

Table 1
Spectral dissociation constants for the interaction of chlorinated hydrocarbons with liver microsomal cytochrome P-450 from variously-treated rats

Compound	Spectral dissociation constant (μM) Control	Phenobarbital-treated	3-methylcholanthrene-treated
Tetrachloromethane	nd[1]	500	nd
1,1,1-trichloroethane	nd	1000	nd
1,1,2-trichloroethane	nd	1000	nd
1,1,2,2-tetrachloroethane	nd	250	nd
Pentachloroethane	179	77 (14)[2]	185
Hexachloroethane	77	48 (8)	83
Tetrachloroethane	278	56 (26)	455

Values are based on duplicate determinations with microsomes from pooled rat livers, 4 to 6 rats in each treatment groups.

[1] indicates not determined.

[2] values in parenthesis are K_S values of 'high' affinity binding site of cytochrome P-450.

enzymes are behind the hydrophobic barrier. This view has been supported [5,6], also with respect to microsomal conjugations [13,14], and challenged by later studies [7–9]. These two views on the role of lipid solubility in drug–microsome interactions need not be entirely contradictory, because one can imagine that a compound must have minimum lipid solubility in order to be metabolizable by the microsomal system and if this requirement is fulfilled, mainly other properties, e.g. stereoselectivity affect the actual interaction of a compound with the microsomal cytochrome *P*-450.

Since we showed that the inducibility of cytochrome *P*-450-linked drug oxidation is inversely correlated with the lipid solubility of barbiturates (with the exception of allyl-barbiturates) [15] we have searched for a suitable series of simple compounds for studying the effect of lipid solubility on drug-cytochrome *P*-450 interactions. Fujita et al. [10] have shown that the addition of chlorine atoms to the carbon skeleton increases lipid solubility of a compound. In a series of chlorine-substituted methanes, ethanes, and ethenes we showed that the more chlorine atoms in the molecule the larger the type I spectral change and the smaller the spectral dissociation constant. The absolute values of interaction parameters (K_s, O.D._{max}) depend on the nature of the microsomal preparation. This may indicate that different kinds of CO-binding hemoproteins reside in the microsomes from variously-treated rat livers. It is interesting that the treatment of rats with 3-methyl-cholanthrene decreases the magnitude of type I spectral changes although it increases the amount of microsomal CO-binding hemoprotein. Despite differences mentioned above the present study seems to support the opinion that an important property of a compound to determine its affinity to cytochrome *P*-450 (it is, K_s) and its extent of interaction (it is O.D._{max}), is lipid solubility. However, this fact does not deny the possible significance of stereochemical factors in the interaction of more complicated compounds with the cytochrome *P*-450.

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