Characterization of the Cytochrome P-450 Species Induced by *trans*-Stilbene Oxide *

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Cytochrome P-450 (E.C. 1.14.14.1) is a key enzyme in the metabolism of xenobiotics and is involved in the first step of the detoxication of a large variety of substances. The cytochrome P-450 system is localized mainly on the endoplasmic reticulum,[1] but occurs also in the nuclear envelope and in mitochondria. This system can be induced by poor substrates and two classical types of inducers have been identified. These are exemplified by phenobarbital [2] and 3-methylcholanthrene,[3] both of which induce the "phase I" activities of the cytochrome P-450 system much more dramatically than "phase II" and "phase III" enzymes.

Recently, a third type of inducer, *trans*-stilbene oxide, (*1*) has also been characterized.[4] This xenobiotic induces the "phase II" enzymes epoxide hydratase and glutathione *S*-transferase as much as 6- and 3-fold, respectively, while only increasing the microsomal content of cytochrome P-450 to about 220 % of the control level. The substrate specificities and nature of the induced enzymes are presently under investigation in our laboratory.

Of interest here is the cytochrome P-450 species induced by treatment of rats with *1*. It is well established that there are several forms of cytochrome P-450 and generally suspected that there are many. The question raised here is whether the cytochrome P-450 species induced by *1* is the same as that induced by phenobarbital or by 3-methylcholanthrene or is it perhaps a new form of the cytochrome? In addition, it was of interest to determine whether *1* can be metabolized by the cytochrome P-450 system, *i.e.*, whether the effect of *1* on this system can be called substrate induction, as is the case for phenobarbital and 3-methylcholanthrene.

Experimental. Male Sprague-Dawley rats weighing 180–200 g were used in these experiments and given free access to food and water. *trans*-Stilbene oxide (400 mg/kg body weight in 1 ml corn oil), phenobarbital (80 mg/kg in isotonic saline), and/or 3-methylcholanthrene (20 mg/kg in corn oil) were injected intraperitoneally into the experimental animals once daily for 5 days, while control rats received corn oil or saline. After starvation overnight the rats were decapitated and the total microsomal fraction prepared from the liver in the usual fashion.[5]

Ethylmorphine deethylation,[6] ethoxycoumarin *O*-deethylation,[7] and benzo[a]pyrene monooxygenase [8] were all assayed using published procedures. In addition, cytochrome P-450, epoxide hydratase, and glutathione *S*-transferase were all measured routinely to assure that induction had been achieved. Cytochrome P-450 was induced in these experiments to 220–250 % of the control level.

Binding spectra were measured with 1 mM *1* by a somewhat modified procedure.[9] The pattern of benzpyrene metabolites was also determined using high performance liquid chromatography according to standard procedures.[10]

Table 1 shows the effects of treating rats with *1*, phenobarbital, and/or 3-methylcholanthrene on three different activities catalyzed by the cytochrome P-450 system. The activities have been expressed per nmol cytochrome P-450 in order to compare the characteristics of this component in the different preparations. It can be clearly seen that the substrate specificity of the cytochrome P-450 system after treatment with *1* resembles that seen after treatment with phenobarbital more closely than that seen after 3-methylcholanthrene induction.

In order to further compare the species of cytochrome P-450 induced by *1* with that induced by phenobarbital the pattern of benzpyrene metabolites produced by microsomes from *1*-treated rats was determined (Fig. 1). It is known that microsomes from phenobarbital-treated rats produce more 4,5-

Table 1. Effects of different inducers on the substrate specificity of the microsomal cytochrome P-450 system. [(ηmol metabolized)/min (ηmol cyt. P-450)].[a]

Inducer	Benzo[*a*]-pyrene	Ethoxy-coumarin	Ethyl-morphine
Control	1.90	2.28	4.90
Phenobarbital	1.79	1.83	3.01
3-Methyl-cholantrene	4.33	3.44	0.61
trans-Stilbene oxide (*1*)	1.50	2.50	1.82
Phenobarbital/3-methylcholantrene/ *trans*-stilbene oxide	–	2.51	1.10

[a] All assays were performed in duplicate with microsomal fractions prepared from three rats. For further details see the experimental section.

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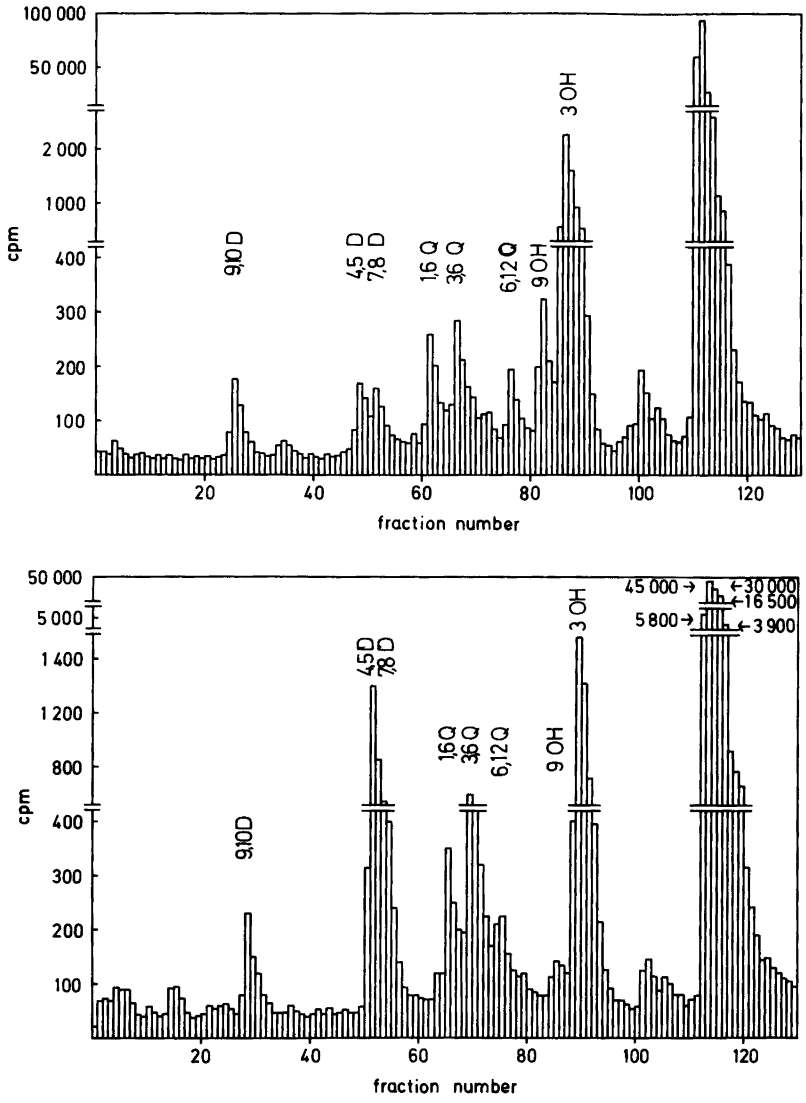

Fig. 1. High performance liquid chromatography elution pattern of benzo[*a*]pyrene metabolites produced by control rat liver microsomes (A) and with microsomes from *1*-treated animals (B). The major metabolites in order of elution are 9,10-dihydroxy-9,10-dihydro- (9,10D), 4,5-dihydroxy-4,5-dihydro- (4,5D), 7,8-dihydroxy-7,8-dihydrobenzo[*a*]pyrene (7,8D), 1,6-benzo[*a*]pyrene quinone (1,6Q), 3,6-benzo[*a*]-pyrene quinone (3,6Q), 6,12-benzo[*a*]pyrene quinone (6,12Q), 9-hydroxy- (9 OH) and 3-hydroxybenzo[*a*]-pyrene (3 OH).

dihydroxy-4,5-dihydrobenzo[*a*]pyrene and less 9,10-dihydroxy-9,10-dihydrobenzo[*a*]pyrene and 9-hydroxybenzo[*a*]pyrene than do microsomes from control animals. Our studies, as well as those by Oesch and coworkers,[11] reveal a very similar change in metabolite pattern after induction with *1*.

Thus, in terms of substrate specificity and of the pattern of benzpyrene metabolites produced, the species of cytochrome P-450 induced by *1* closely resembles the species induced by phenobarbital. We are presently determining whether the cytochrome P-450 from *1*-treated animals can be inhibited or precipitated by antibodies to cytochrome P-450 purified from phenobarbital-treated rats.

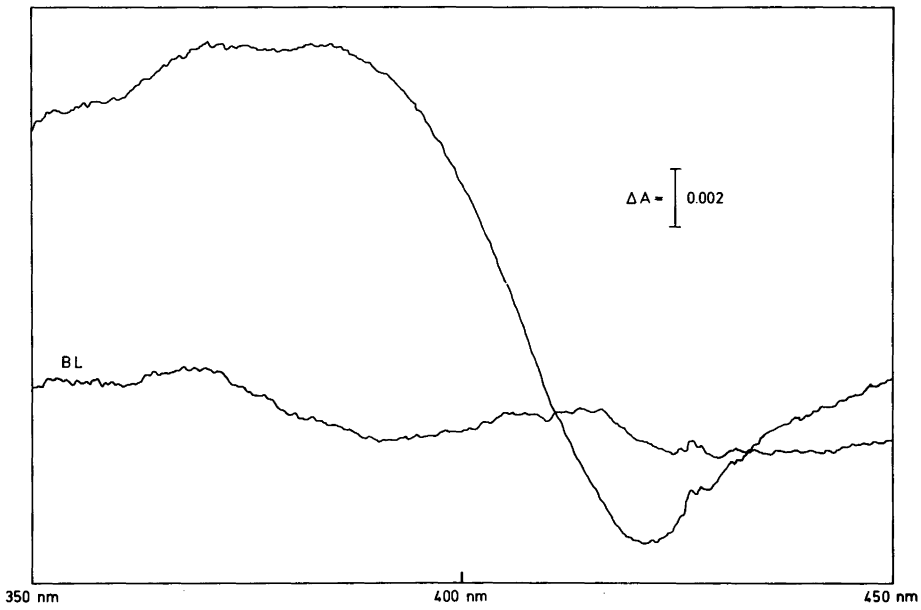

Fig. 2. Difference spectrum of *1* with microsomes from *1*-treated rats.

One aspect in which the phenobarbital-induced cytochrome P-450 seems to differ from the *1* induced form is in ability to bind *1*. Binding was monitored by looking for the appearance of a typical binding spectrum. As is shown in Fig. 2, such a spectrum can be seen using microsomes from *1*-treated rats. This spectrum is even more pronounced with microsomes isolated after induction with 3-methylcholanthrene. However, with control microsomes and microsomes prepared after administration of phenobarbital we have not yet succeeded in obtaining a binding spectrum with *1*. This difference requires further investigation.

The binding spectrum shown in Fig. 2 is a typical type I spectrum, *i.e.*, the type of spectrum seen with compounds which can be metabolized *via* the cytochrome P-450 system. This suggests that *1* is indeed a substrate for the cytochrome P-450 system, which can presumably catalyze ring hydroxylation of this substance.[12] If further investigations support this conclusion, then the induction of cytochrome P-450 by *1* is also substrate induction.

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