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DRUG METABOLISM BY THE HUMAN HEPATOMA CELL, Hep G2

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SUMMARY: The human liver-derived cell line, Hep G2, has aryl hydrocarbon hydroxylase and 7-ethoxycoumarine o-deethylase activities. Partial purification of cytochrome P-450 from Hep G2 cells provided spectral evidence of this hemeprotein in the purified fraction. These results suggest that Hep G2 cells will be useful for the study of cytochrome P-450 and the regulation of mixed function oxidase activities in liver cells of human origin.

Our aim in this study was to examine cytochrome P-450-mediated drug metabolism in human liver. Since it is difficult to obtain fresh human liver for experimental purposes, we investigated the possibility of using a model tissue culture system. We elected to utilize the liver-derived human cell line, Hep G2 (1), which exhibits numerous parenchymal cell functions including metabolism of polycyclic aromatic hydrocarbons, e.g., benzo[a]pyrene (2,3) and has been reported to contain a P-450 isozyme which reacts with an antibody against phenobarbital-inducible rat liver P-450 PB_2 (4).

MATERIALS AND METHODS

Cell cultures: Hep G2 cells, obtained from Dr. B. B. Knowles of the Wistar Institute of Anatomy and Biology, Philadelphia, PA, were grown in Eagles Minimum Essential Medium (GIBCO, Grand Island, N.Y.) supplemented with 10 % defined calf bovine serum (HyClone, Logan UT). Cultures were passaged at 5-7-day intervals by trypsinization followed by reseeding at a 1:3 dilution in growth medium. Cells were collected by trypsinization, washed once with Earle's buffer salt solution and cell pellets were stored in liquid nitrogen until use.

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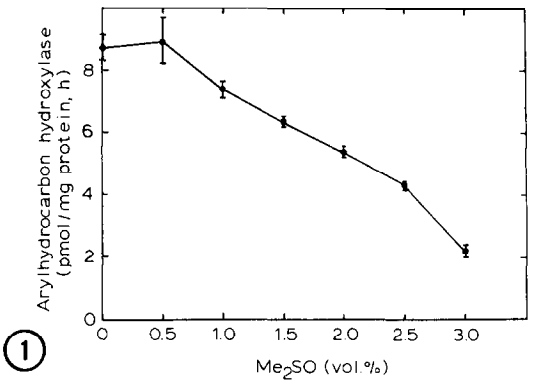
Enzyme assays: Aryl hydrocarbon hydroxylase activity (5) and 7-ethoxycoumarine de-ethylase activity (6) were assayed fluorometrically, using freeze-thawed (3 cycles) cell homogenates. Cytochrome P-450 content was determined according to Omura and Sato (7) and protein concentration was determined by the dye binding assay (8).

Partial purification of cytochrome P-450: Hep G2 cells (~45gm of packed cells, collected and stored over 2 years in liquid nitrogen) were suspended in 3 vol of 0.1M potassium phosphate (KPi) buffer (pH 7.4) supplemented with 7mM 2-mercaptoethanol, 0.1mM $ZnSO_4$, 0.5mM phenylmethylsulfonyl fluoride and 1mM benzamidine. Cells were sonicated in a Braunsonic 1510 sonicator at a setting of 100 Watts for 8 x 10sec bursts and centrifuged at 18,000 x g, for 20 min. The supernatant fraction from the 18,000 x g centrifugation was collected and centrifuged at 104,000 x g for 60 min. Microsomes were obtained as pellets from the 104,000 x g centrifugation. Microsomes were homogenized in 0.1M KPi buffer (pH 7.4)/20%(w/v) glycerol/1mM ethylenediaminetetraacetic acid(EDTA)/1mM dithiothreitol(DTT). Cholic acid solution (20% w/v, pH 7.4) was added to microsomes to give a final concentration of 1.0 %(w/v). The mixture was stirred for 20 min at 4°C. Polyethylene glycol 8000 (PEG) (J.T. Baker, Phillipsburg, N.J.) was added to give a final concentration of 9%(w/v). After stirring for 30 min at 4°C, the mixture was centrifuged at 38,000 x g, for 20 min. The PEG solution was added to the supernatant to give a final concentration of 17%(w/v) and the mixture was stirred for 20 min at 4°C. The mixture was centrifuged at 38,000 x g, for 20 min. The pellets ("9-17% PEG fraction") were collected which contained cytochrome P-450 (9), and dissolved in 10 ml of buffer A [10mM KPi(pH 7.4)/20%(w/v) glycerol/1.0mM EDTA/1.0mM DTT/0.1%(w/v) cholic acid/0.2%(w/v) Emulgen 911(Kao-Atlas, Tokyo)]. The mixture was centrifuged at 10,000 x g, for 15 min. Then the supernatant fraction was applied onto a column of DE52 (Whatman, Clifton, N.J.) (ϕ1.5 x 30 cm) which had been equilibrated with Buffer A. The column was washed with 50 ml of Buffer A. Cytochrome P-450 was eluted from the column with Buffer A containing 0.3M NaCl.

RESULTS

Aryl hydrocarbon hydroxylase and 7-ethoxycoumarine de-ethylase activities: Hep G2 cells contain readily detectable levels of aryl hydrocarbon hydroxylase (Fig. 1) and 7-ethoxycoumarine de-ethylase (Fig. 2) activities. We showed earlier that the syntheses of 5-aminolevulinic acid dehydratase and heme in Hep G2 cells are increased in response to dimethylsulfoxide (Me_2SO) treatment (10). Therefore we examined in this study whether or not cytochrome P-450-mediated enzyme activities are also influenced by Me_2SO treatment. As shown in Fig. 1 and 2, aryl hydrocarbon hydroxylase activity decreased and 7-ethoxycoumarine de-ethylase



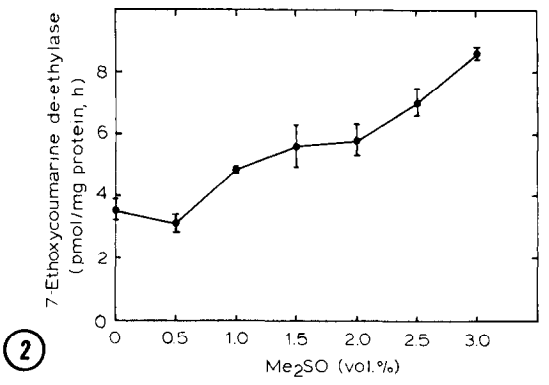
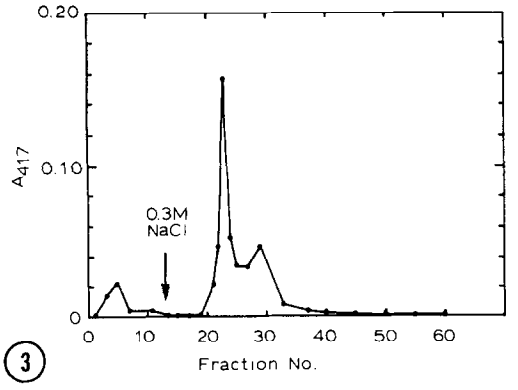


Figure 1. Aryl hydrocarbon hydroxylase activity in Hep G2 cells. Enzyme activity was assayed using whole homogenates of Hep G2 cells which had been incubated with Me_2SO for 96h. Data are the mean ± S.E. of triplicate determinations.

Figure 2. 7-Ethoxycoumarine de-ethylase activity in Hep G2 cells. Enzyme activity was assayed using the same homogenates as in Figure 1. Data are the mean ± S.E. of triplicate determinations.

activity increased in response to Me_2SO treatment. The reason for the discrepant induction responses of the two P-450 dependent mixed function oxidases is unclear, but it has also been demonstrated that these two enzyme activities may exhibit disparate induction responses in rat liver following treatment with polychlorinated biphenyls (11).

Cytochrome P-450: The elution profile of cytochrome P-450 from DE52 column chromatography is shown in Fig. 3. Three hemeprotein peaks, one major and two minor, were detected as judged by the absorption at 417nm; only the major peak contained cytochrome P-450. Cytochrome P-450 was not detectable spectrally using non-purified microsomes (Fig. 4), while it was clearly detectable in the major peak of the partially purified fraction (Fig. 4). If one assumes that the recovery of cytochrome P-450 after PEG treatment and DE52 chromatography is about 15 % (12), the total amount of cytochrome P-450 and its specific content in the microsomes would be 2.1 nmoles, and 2.5 pmol/mg protein, respectively. Since the specific content of P-450 in the purified fraction was 40



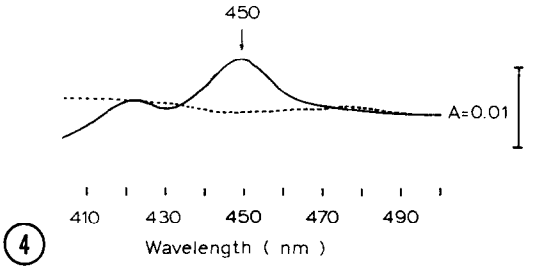


Figure 3. A chromatographic elution profile of cytochrome P-450 from a DE52 cellulose column. Chromatographic conditions are described in Materials and Methods.

Figure 4. Reduced CO-difference spectra of the partially purified cytochrome P-450 from Hep G2 cells. Purified P-450 (———); cholate-solubilized microsomes (-------).

pmol/mg protein (Table I), it can be reasonably concluded that P-450 was purified at least 16-fold. Western blot analyses using antibodies against purified rat liver cytochrome $P\text{-}450_b$, $P\text{-}450_c$ and $P\text{-}450_{C\text{-}M/F}$(12) revealed no corresponding isozymes either in microsomes or in a highly purified P-450 fraction from Hep G2 cells.

DISCUSSION

The results of this study demonstrate that Hep G2 cells, a liver-derived human cell line, exhibit active aryl hydrocarbon hydroxylase and 7-ethoxycoumarine de-ethylase activities, both of

TABLE I. Purification of cytochrome P-450 from Hep G2 cells

Fraction	Protein	Cytochrome P-450	Specific Content of P-450
	mg	nmol	nmol/mg protein
Microsomes	840	n.d.	n.d.
DE52	7.8	0.32	0.04

Microsomes were isolated from 45gm of packed Hep G2 cells as described in Materials and Methods. Cytochrome P-450 was not detectable in microsomes.

which are cytochrome P-450-dependent mixed function oxidase activities. In contrast, ethylmorphine N-demethylase and aniline hydroxylase activities were undetectable in Hep G2 homogenates(data not shown). Our data on aryl hydrocarbon hydroxylase are consistent with the earlier finding that Hep G2 cells are capable of metabolic activation of benzo[a]pyrene (3). 7-Ethoxycoumarine de-ethylase activity has also been demonstrated in untreated Hep G2 cells (4). These data not only confirm that Hep G2 cells are active in the biotransformation of aromatic hydrocarbons and other drug substrates, but also indicate that such activities respond to the *in vitro* addition of perturbants such as Me_2SO.

Although mixed function oxidase activities have previously been reported in Hep G2 cells, no spectral evidence for the existence of cytochrome P-450 has earlier been provided. Our study showed that while a typical CO-difference spectrum of cytochrome P-450 was undetectable in microsomes after initial isolation from Hep G2 cells, the typical CO-spectrum of the cytochrome can be demonstrated following partial purification. Our data suggest that the specific content of cytochrome P-450 in Hep G2 cells is 2.5 pmol/mg microsomal protein.

Dawson et al. (4) reported that two protein bands can be detected in 30 μg of Hep G2 cells using an antibody against rat liver cytochrome P-450 PB_2, and concluded that these proteins were P-450 isozymes which were equivalent to a phenobarbital-inducible rat liver P-450 isozyme, despite the fact that these proteins had unusually high molecular weights for cytochrome P-450, i.e., 62 Kd and 64 Kd. Using an equivalent antibody against the phenobarbital-inducible form of rat liver cytochrome P-450, and the purified P-450 fraction from Hep G2 cells, we could not detect any P-450 isozymes by Western blot analyses. The reason for this is not clear, but based on our calculation of the P-450 content, we

conclude that cytochrome P-450 in non-purified Hep G2 microsomes is below the detection limit by Western blot analysis.

The regulation of cytochrome P-450 and its associated mixed function oxidases in human liver-derived cells such as Hep G2 is clearly important for a better understanding of the properties and regulation of human drug metabolism. Such a model system which also responds to *in vitro* chemical additions should prove valuable in the study of xenobiotic biotransformations, zenobiotic-zenobiotic interactions and their attendant benefits or risks to man.

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