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Characterization of Human Lung Microsomal Cytochrome P-450 1A1 and Its Role in the Oxidation of Chemical Carcinogens

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SUMMARY

Rat and human lung microsomal cytochrome P-450 (P-450) enzymes have been characterized with regard to their catalytic activities towards several xenobiotic chemicals, including procarcinogens, in different microsomal preparations. Rat lung microsomal P-450s were more active than the human P-450s in catalyzing most of the monooxygenation reactions. Human lung microsomal P-450 was solubilized and purified. Human lung microsomes contain ~10 pmol of P-450/mg of protein, on the basis of $Fe^{2+}\cdot CO$ versus Fe^{2+} difference spectra of the eluates obtained from an octylamino-agarose column. The partially purified P-450 preparations from two human lung microsomal samples showed high activities for the conversion of both (+)- and (−)-isomers of 7,8-dihydroxy-7,8-dihydrobenzo(*a*)pyrene to genotoxic products. After DEAE-cellulose column chromatography, a partially purified P-450 fration containing polypeptides of M_r 52,000 and 58,000 was obtained from the early fraction of the octylamino-agarose column eluate, and an electrophoretically homogeneous protein having a molecular weight of ~52,000 was recovered from a latter fraction. The amino-terminal amino acid sequences of the two peptides in the earlier fraction were determined; neither polypeptide appears to resemble any known P-450 protein. The protein from the latter octylamino-agarose fraction was immunoreactive with anti-rat P-450 1A2 and anti-human P-450 1A2 but not with antibodies raised against other P-450 enzymes or autoimmune antibodies that specifically recognize human P-450 1A2. A tryptic peptide was isolated from the preparation, and the amino acid sequence matched that of human P-450 1A1 perfectly (residues 31–48) but not that of human P-450 1A2. All of nine human lung microsomal samples examined contained proteins that were immunoreactive with rabbit anti-rat P-450 1A2 and catalyzed the activation of 7,8-dihydroxy-7,8-dihydrobenzo(*a*)pyrene. The activities could be inhibited by rabbit anti-rat P-450 1A2 and, to a lesser extent, by anti-rat P-450 1A1. The addition of 7,8-benzoflavone caused inhibition or stimulation, depending upon the particular human lung microsomal preparation. Thus, this work clearly shows that human lung microsomes contain at least two major P-450 enzymes; human P-450 1A1 is present in lungs and can actually catalyze the activation of environmental procarcinogens, including polycyclic aromatic hydrocarbons.

P-450[2] enzymes in the lung are of interest because this tissue is an early site of entry into the body for many chemical carcinogens. Further, there are several toxicants and carcinogens that selectively cause lung injury, regardless of their site of administration (8). In order to understand the etiology of lung cancer, it is necessary to delineate the contributions of enzymatic activation of carcinogens at the site of tissue damage and also at major extra-target sites, such as the liver.

The study of pulmonary P-450 enzymes is problematic because of their low abundance, relative to their hepatic counterparts, as well as the complexity of cell types in the lung (9, 10). Rabbit lung has been a popular experimental model because the total amount of P-450 appears to be considerably greater than that in many other animals (11). The major P-450s present

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[2] The P-450 enzymes characterized in this laboratory are sometimes referred to with abbreviations based upon the substrates that were utilized during purification (1). See Nebert *et al.* (2) for classification of the P-450 genes and Guengerich (1) for review of individual human P-450 enzymes; both references describe related preparations characterized in other laboratories. Briefly, P-450 3A4 ($P\text{-}450_{NF}$) is the nifedipine oxidase (3, 4), P-450 2D6 ($P\text{-}450_{DB}$) is the debrisoquine 4-hydroxylase/bufuralol 1′-hydroxylase (5), P-450 1A2 ($P\text{-}450_{PA}$) is the high affinity phenacetin *O*-deethylase (5, 6), and P_1-450 (P-450 1A1) is the product of the *CYP 1A1* gene (7).

ABBREVIATIONS: P-450, cytochrome P-450; SDS, sodium dodecyl sulfate; PB, phenobarbital; BNF, β-naphthoflavone (5,6-benzoflavone); ISF, isosafrole; MeIQ, 2-amino-3,5-dimethylimidazo[4,5-*f*]quinoline; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; Trp P-2, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; 2-AA, 2-aminoanthracene; BA-3,4-diol, (*trans*)-3,4-dihydroxy-3,4-dihydrobenzo(*a*)anthracene; BFA-9,10-diol, (*trans*)-9,10-dihydroxy-9,10-dihydrobenzo(*b*)fluoranthene; DMBA-3,4-diol, (*trans*)-3,4-dihydroxy-3,4-dihydro-7,12-dimethylbenz(*a*)anthracene; B(*a*)P, benzo(*a*)pyrene; B(*a*)P-7,8-diol, 7,8-dihydroxy-7,8-dihydrobenzo(*a*)pyrene; AFB_1, aflatoxin B_1; Trp P-1, 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole.

appear to be those now termed P-450s 1A1, 2B4, and 4B1 (11, 12). The former of these is inducible by polycyclic aromatic hydrocarbons and related compounds. These three P-450s have all been purified, and their roles in the metabolism of a number of drugs and carcinogens have been explored. However, very little pulmonary bioassay work has been done with carcinogens in rabbits. A considerably larger database exists for carcinogenesis in rodents, although the pulmonary P-450s appear to be less plentiful and have not been as well characterized. P-450 1A1 is present in the lungs of untreated rats at only low levels but is highly inducible by polycyclic hydrocarbons; P-450 1A2 appears to be present at low levels and is rather refractory to induction (13). P-450 2B1, an ortholog of rabbit P-450 2B4, is a major constitutive rat lung enzyme and is not inducible in the lung (14, 15). P-450 2A3 and a P-450 3A family member are indicated by cDNA cloning (16) and immunochemical (17) experiments, respectively. A P-450 2F member is probably also present (18). Other work suggests that hamsters contain inducible lung P-450 1A1 (19) and that hamsters and guinea pigs have lung P-450 4B enzymes (20).

There exists considerable interest in human lung P-450s because of their possible involvement in the etiology of lung cancer, particularly that associated with tobacco. Until recently (21), a distinctive $Fe^{2+}\cdot CO$ versus Fe^{2+} difference spectrum of human lung P-450 had not been recorded, and uncertainly exists as to what levels of P-450 actually exist in human lung; several lines of evidence (e.g., P-450 spectra and enzyme activities) strongly suggest that P-450 levels are considerably lower in human than in rabbit or rat lung microsomes. To date, no human lung P-450 has been purified, but other lines of evidence indicate that P-450s 1A1, 2F1, and 4B1 are present (18, 22, 23); a P-450 3A enzyme may also exist, as suggested by (low) nifedipine oxidation activity that can be inhibited by a specific antibody (24). Kellerman *et al.* (25) suggested that susceptibility to lung cancer may be linked to polymorphic inducibility of (lymphocyte) B(*a*)P hydroxylation, apparently catalyzed by P-450 1A1, but this hypothesis remains unproven; however, a correlation does seem to exist between basal levels of the enzyme and incidence of lung cancer in smokers (26). A relationship between lung cancer susceptibility and debrisoquine 4-hydroxylation has been observed (27), although the extent of expression of the enzyme (P-450 2D6) in lung tissue has not been reported.

In order to better understand how lung P-450s metabolize chemical carcinogens, a series of studies was carried out to compare some catalytic activities of human lung microsomes with each other and with rat lung and liver microsomes. A protein that appears to be P-450 1A1 was purified to electrophoretic homogeneity from human lung microsomes.

Materials and Methods

Chemicals. Polycyclic hydrocarbon derivatives were obtained from the National Cancer Institute Repository, through Midwest Research Institute (Kansas City, MO). Other procarcinogens and chemicals used were from the same sources described previously (28).

Enzyme preparations and antibodies. Human lung samples were obtained from tissue donors through the Cooperative Human Tissue Network, University of Alabama (Birmingham, AL), and microsomal fractions were prepared as described elsewhere (29). Human liver samples were obtained from organ donors through Tennessee Donor Services (Nashville, TN). Male Sprague-Dawley rats (250 g; Harlan Industries, Indianapolis, IN) were treated intraperitoneally with each of several P-450 inducers: PB (80 mg/kg, daily for 3 days), BNF (50 mg/kg, daily for 3 days), or ISF (100 mg/kg, daily for 3 days). Rats were fasted overnight before killing, and liver and lung microsomes were prepared as described elsewhere (29) and suspended in 10 mM Tris·HCl buffer (pH 7.4) containing 0.1 mM EDTA and 20% (v/v) glycerol (at a concentration of 5 g of lung tissue equivalent/ml).

Human P-450 3A4 (3) and P-450 1A2 (5) were prepared as described elsewhere. Rat P-450s 1A1 and 1A2 (30) and rabbit P-450 1A2 (31) were purified to electrophoretic homogeneity as described previously. Rabbit anti-human P-450 3A4 (3), anti-rat P-450 1A1 (30), and anti-rat P-450 1A2 (30) preparations have been characterized elsewhere. The anti-rat P-450 1A1 antiserum was cross-adsorbed with microsomes prepared from livers of PB-treated rats (immobilized on gels), to remove antibodies reacting with rat P-450 1A2 (14). IgG fractions were prepared and used to inhibit catalytic activities in microsomes (14). Rabbit liver NADPH-P-450 reductase was purified using the general procedure described by Yasukochi and Masters (32).

Human P-450 1A1 and P-450 1A2 enzymes were expressed in the yeast *Saccharomyces cerevisiae*, using adaptations of the approaches presented elsewhere (33). The cDNA sequences of the inserts were both verified, and further details and use of these constructs will be presented elsewhere.[3] Autoimmune antibodies [from a patient (coded NAL) who had been treated with dihydralazine] are specific to human P-450 1A2 (34) (shown not to recognize recombinant human P-450 1A1 in immunoblotting analysis).

Purification of human lung P-450s. Human lung microsomes from samples HLu-9 (360 mg of microsomal protein/75 g of lung tissue) and HLu-10 (400 mg of protein/100 g of lung tissue or 1600 mg of protein/330 g of lung tissue) were solubilized with 0.7% (w/v) sodium cholate, in a buffer containing 100 mM potassium phosphate (pH 7.4), 1 mM EDTA, 1 mM dithiothreitol, and 20% (v/v) glycerol. The fraction precipitating between the addition of 5 and 16% polyethylene glycol 8000 was obtained and used for further chromatography. The pellets thus obtained were solubilized in the buffer containing 0.7% sodium cholate. The solubilized supernatants were applied to aminooctyl-Sepharose 4B columns (1.6 × 16 cm), as described previously (29). After washing of the columns with the same buffer containing 0.5% (w/v) sodium cholate, the P-450 fractions were recovered with buffer containing 0.4% (w/v) sodium cholate and 0.2% (w/v) Emulgen 911. The eluates were divided into two fractions; the former fraction contained P-450 enzymes not cross-reactive with anti-rat P-450 1A2, and the latter fraction contained a protein highly reactive with anti-P-450 1A2. Both fractions were subjected separately to DEAE-Sephacel column chromatography (Pharmacia, Piscataway, NJ). The column (1.6 × 5 cm) was equilibrated with 5 mM potassium phosphate buffer (pH 7.5) containing 0.1 mM EDTA, 0.1 mM dithiothreitol, 0.2% (w/v) Emulgen 911, and 20% (w/v) glycerol. The column was washed extensively, and the P-450 fraction was eluted with 200 ml of a linear gradient of 0 to 0.5 (or 1.0) M NaCl.

Assays. 7-Ethoxyresorufin and 7-pentoxyresorufin *O*-dealkylation (35), B(*a*)P 3-hydroxylation (29), and phenacetin *O*-deethylation (36) were determined as described elsewhere. Protein concentrations were estimated using the Pierce bincinchonic acid procedure, according to the manufacturer's directions (Pierce Chemical Co., Rockford, IL). P-450 was estimated spectrally from $Fe^{2+}\cdot CO$ versus Fe^{2+} difference spectra, as described by Omura and Sato (37). Protein immunoblotting was done as described elsewhere (14).

Details of the *umu* gene expression assay for DNA damage are described elsewhere (28, 38); in general, human and rat lung microsomes, containing 10–50 μg of protein, were used with 10 μM procarcinogen concentrations, in a volume of 1.0 ml of 50 mM potassium phosphate buffer (pH 7.4) containing an NADPH-generating system (consisting of 5 mM glucose 6-phosphate, 0.5 mM $NADP^+$, and 1 IU

[3] J.-C. Gautier and P. Beaune, and D. Pampon, Yeast coexpression of human P-450 1A1 and microsomal epoxide hydrolase as tools for the analysis of the first steps of benzo(*a*)pyrene activation, manuscript in preparation.

yeast glucose 6-phosphate dehydrogenase/ml) and a suspension of bacterial tester strain *Salmonella typhimurium* TA1535/pSK1002, as described previously (28, 38). In the reconstituted systems, microsomes were replaced with a premixed complex of (final concentrations in assay) 0.01–0.20 μM P-450, 0.05 μM rabbit NADPH-P-450 reductase, and 7.5 μM L-α-1,2-dilauroyl-*sn*-glycero-3-phosphocholine. The expression of the *umu* gene was monitored by measuring β-galactosidase activity (the product of the fused *umuC″lacZ* gene), and the bioactivation is presented as units of β-galactosidase activity/min/mg of protein or as units/min/nmol of P-450 (28, 38).

The high performance liquid chromatography procedures used in peptide isolation and amino acid sequence analysis are described in detail elsewhere (39, 40) and were used in this work with only slight modification. Recovery of phenylthiohydantoin derivatives was calculated from comparison with external standards. In the case of the amino-terminal amino acid sequence determinations, the resolved polypeptides (SDS-polyacrylamide gel electrophoresis) were electrophoretically transferred to a sheet of polyvinylidenefluoride and were handled according to the manufacturer's directions (Applied Biosystems, Foster City, CA); the Applied Biosystems 470A sequenator was optimized for analysis of blotted samples.

Results

Catalytic activities of rat and human lung microsomes. Nine human lung microsomal samples were prepared, and catalytic activities were measured with several compounds; the rates were compared with those of human liver microsomes, rat lung microsomes, and rat liver microsomes (Table 1). B(*a*)P 3-hydroxylation and ethyoxyresorufin *O*-deethylation activities (known to be catalyzed by P-450 1A1 in rat liver) (30) were detected in human lung microsomes, but these activities were usually very low, compared with other activities in rat lung and rat and human liver microsomes. Rates of activation of (+)-B(*a*)P-7,8-diol correlated well with the rates of (−)-B(*a*)P-7,8-diol activation ($r = 0.87$) and ethoxyresorufin *O*-deethylation ($r = 0.79$). Also, rates of ethoxyresorufin *O*-deethylation correlated with B(*a*)P 3-hydroxylation ($r = 0.71$) in different human lung preparations.

Activities of human lung microsomal samples HLu-8 and HLu-9 towards several procarcinogens are compared in Table 2; the former sample contained none of the black color associated with smoking-associated deposits (21), whereas the latter sample contained a considerable amount (information from the Cooperative Human Tissue Network indicated that both samples were from cigarette-smokers). The results indicate that human lung microsomes can catalyze the activation of BFA-9,10-diol, DMBA-3,4-diol, 2-AA, and AFB_1, as well as B(*a*)P-7,8-diol. Lung microsomes from sample HLu-9 had much higher activities for the activation of several procarcinogens than did those prepared from sample HLu-8, except for AFB_1. However, all of these catalytic activities were considerably lower than those measured in lung microsomes of untreated or BNF-treated rats.

Purification of human lung microsomal P-450s. Human lung microsomes (sample HLu-10) were solubilized, fractionated with polyethylene glycol 8000, resolubilized, and subjected to octylamino-Sepharose 4B column chromatography, as described in Materials and Methods. P-450 was monitored by measuring A_{417} (Fig. 1), and immunoblotting was done on the fractions using rabbit anti-rat P-450 1A2. Fraction A contained P-450 proteins that did not cross-react with the antibodies, whereas fraction B contained a protein that was highly reactive with anti-rat P-450 1A2 (results not shown). These two fractions were loaded onto separate DEAE-Sephacel columns, and P-450 fractions were eluted with linear gradients of 0 to 0.5 (or 1.0) M NaCl (Fig. 2). SDS-polyacrylamide gel electrophoresis indicated that the proteins that eluted in the salt gradient from octylamino-Sepharose fraction A contained partially purified polypeptides of M_r 52,000 and 58,000; the latter protein that eluted in the salt gradient from the octylamino-Sepharose

TABLE 1

Catalytic activities of lung and liver microsomes prepared from rats and humans

The values are presented as means of duplicate or triplicate assays. When no values are shown, no assay was done.

Microsomal sample	Oxidation activity				*umu* response	
	B(*a*)P	7-Ethoxyresorufin	7-Pentoxyresorufin	Phenacetin	(+)-B(*a*)P-7,8-diol	(−)-B(*a*)P-7,8-diol
	pmol of product/min/mg of protein				*units/min/mg of protein*	
Human lung						
HLu-2	0.81	0.56	0.47	<1	5.6	4.3
HLu-8	0.04	0.35	0.69	<1	3.8	1.4
HLu-9	0.74	0.77	1.20	<1	10.8	6.1
HLu-10	1.06	0.36	1.25	<1	4.2	2.8
HLu-11	0.92	0.85	1.23	<1	4.1	0.3
HLu-12	1.91	1.26	1.59	<1	9.7	5.9
HLu-13	1.16	0.60	1.40	<1	1.9	1.6
HLu-14	1.05	0.18	0.54	<1	0.4	2.0
HLu-15	0.07	0.06	0.80	<1	0.3	0.8
Human liver						
HL 101	31.7	50.4	7.1	90	63	
HL 105				42	76	
HL 115	127	24	3.6		151	
Rat lung						
Untreated	11	11	2.5	7.0	48	
PB-treated	15	10	3.8			
BNF-treated	73[a]	54[a]	1.5	4.0	148[a]	
Rat liver						
Untreated	306	31.2	3.4	17	302	
PB-treated	690[a]	172[a]	216[a]	25		
BNF-treated	1330[a]	21,150[a]	24[a]	146[a]	1503[a]	

[a] Significantly greater than value measured in tissue of untreated animals ($p < 0.05$).

TABLE 2

Activation of several procarcinogens by rat and human lung microsomal samples HLu-8 and HLu-9

	Activation of procarcinogen			
	Human lung microsomes[a]		Rat lung microsomes	
	HLu-8	HLu-9	Untreated	BNF-treated
	umu units/min/mg of protein			
(+)-B(*a*)P-7,8-diol	2.8 ± 0.6	14.1 ± 0.4	42	164
(−)-B(*a*)P-7,8-diol	1.1 ± 0.9	4.4 ± 0.1	37	127
BA-3,4-diol	<0.1	<0.1	0.7	6.1
BFA-9,10-diol	<0.1	0.8 ± 0.2	4	46
DMBA-3,4-diol	1.1 ± 0.1	5.8 ± 1.9	17	93
2-AA	1.0 ± 0.1	1.5 ± 0.5		
Trp P-2	<0.1	<0.1	5	41
MeIQ	<0.1	<0.1	44	49
IQ	<0.1	<0.1	10	17
AFB_1	1.3 ± 0.8	0.9 ± 0.1	39	37

[a] The values presented are means ± standard deviations (range) of duplicate experiments.

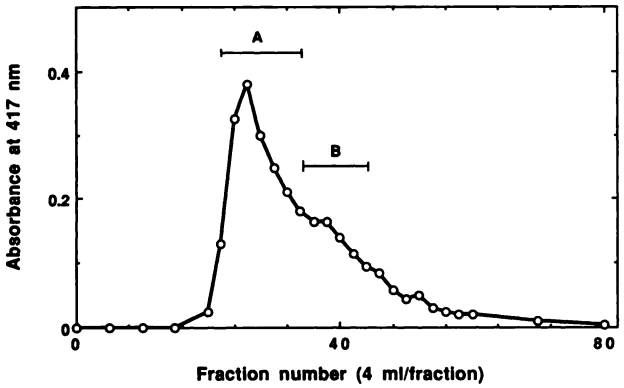

Fig. 1. Elution profile of human lung P-450 by octylamino-Sepharose 4B chromatography. Proteins A and B were pooled as indicated, for further purification.

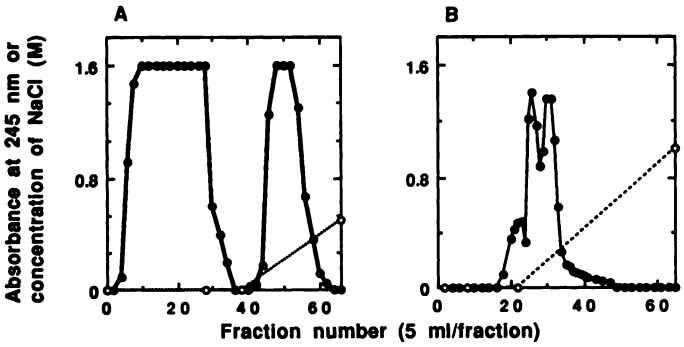

Fig. 2. DEAE-cellulose chromatography of fractions A and B recovered from octylamino-Sepharose 4B chromatography. See Materials and Methods for details. – – –, NaCl gradients. Fractions 31–35 of the material shown in B were pooled on the basis of electrophoretic purity and constitute the P-450 1A1 fraction analyzed (Figs. 3 and 4; Table 3).

fraction B contained a highly purified P-450 of M_r 52,000 (Fig. 3). As discussed below, the apparent denaturation of the M_r 52,000 P-450 enzyme prevented an accurate estimate of its recovery in this step.

Characterization of purified human lung microsomal P-450s. The P-450s were characterized using antibodies raised against rat P-450s 1A1 and 1A2. (The anti-rat P-450 1A1 antibodies used here were highly specific after cross-adsorption with liver microsomes prepared from PB-treated rats, to remove

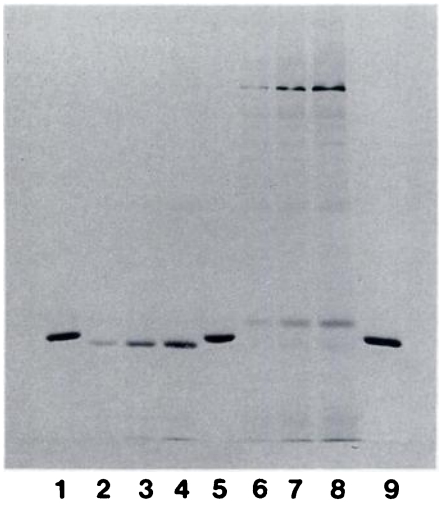

Fig. 3. SDS-polyacrylamide gel electrophoresis of purified human lung and other proteins. *Lanes 1, 5,* and *9*, rabbit liver P-450 1A2 (0.8 μg of protein); *lanes 2, 3,* and *4*, human lung P-450 1A1 (0.2, 0.4, and 0.6 μg of protein, respectively); *lane 5*, rabbit liver P-450 1A2; *lanes 6, 7,* and *8*, a partially purified human lung fraction (octylamino-Sepharose fraction A, Fig. 1; DEAE-Sephacel fractions 45–57, Fig. 2) (0.6, 1.2, and 1.8 μg of protein, respectively). Proteins were stained using ammonical Ag^+ (41).

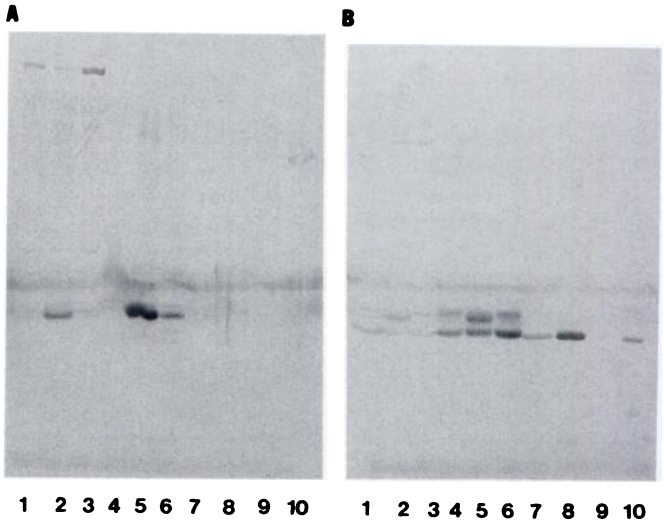

Fig. 4. SDS-polyacrylamide gel electrophoresis and immunostaining of rat and human liver and lung microsomal proteins. The indicated lanes contained ~100 and 10 μg of lung (*lanes 1–3*) and liver microsomal protein (*lanes 4–6*), respectively, prepared from untreated rats (*lanes 1* and *4*) or rats treated with βNF (*lanes 2* and *5*) or ISF (*lanes 3* and *6*), 500 μg of human lung microsomal protein (from sample HLu-10) (*lane 7*), 1 μg of purified human lung P-450 1A1 (*lane 8*), 2 μg of a partially purified human lung P-450 (*lane 9*) (see Fig. 3; octylamino-Sepharose fraction A, Fig. 1; DEAE-Sephacel fractions 45–57, Fig. 2), or 15 μg of human liver sample HL 101 microsomal protein (*lane 10*). Proteins were transferred to nitrocellulose sheets, and immunochemical staining was done with either rabbit anti-rat P-450 1A1 (A) or rabbit anti-rat P-450 1A2 (B).

antibodies that react with rat P-450 1A2.) The highly purified preparation from human lung microsomes (from octylamino-Sepharose 4B fraction B) (Fig. 1) reacted strongly with anti-rat P-450 1A2, but the partially purified preparation (from octylamino-Sepharose 4B fraction A) did not (Fig. 4). This antibody preparation also recognized rat lung microsomal P-450s, as well as rat liver microsomal P-450s and P-450 1A2

protein in human liver microsomes (sample HL 101). In rat lung microsomes there were two proteins that reacted with anti-rat P-450 1A2; the lower molecular weight polypeptide was considered to be P-450 1A2, and only the one of higher molecular weight (P-450 1A1) was induced by the treatment with BNF. Interestingly, anti-rat P-450 1A1 did not recognize the lower molecular weight P-450 proteins in human and rat lung and liver microsomal preparations. This antibody only reacted with rat P-450 1A1, which is inducible by BNF in rats. It was possible to achieve some resolution between recombinant human P-450 1A1 and P-450 1A2 by increasing the acrylamide concentration in the gel (Fig. 5). Rabbit anti-rat P-450 1A2 recognized both of these proteins. Comparison of the lung protein purified here with purified human liver P-450 1A2 and the recombinant proteins indicated that the lung protein is P-450 1A1. Further, the autoimmune antibody preparation was shown to recognize only human P-450 1A2 and not human P-450 1A1. This antibody reacted mainly with liver P-450 1A2 but not at all with the purified lung P-450 (1A1) (results not shown).

An aliquot of the purified protein isolated from human microsomal sample HLu-10 (from octylamino-Sepharose 4B fraction B) was alkylated and partially digested with trypsin (amino-terminal analysis was not successful). A well resolved peptide eluted from the high performance liquid chromatography column was subjected to amino acid sequence analysis (Table 3). This particular peptide had two internal lysine residues and gave excellent repetitive yields at all steps. The sequences of human P-450s 1A1 and 1A2 are ~80% identical (7, 42), and the amino acid sequence of the isolated peptide exactly matched that predicted for codons 31–48 of P-450 1A1. In this particular peptide there are three differences from P-450 1A2, and at each of these cycles the expected P-450 1A1 residue was recovered in good yield.

The partially purified HLu-10 protein sample (octylamino-Sepharose fraction A, DEAE-Sephacel peak eluted with NaCl; Figs. 2 and 3) contained P-450 and cytochrome P-420. The two major bands in the molecular weight range characteristic of P-450s (Fig. 3) were transferred to a polyvinylidenefluoride membrane and subjected to amino-terminal amino acid sequence determination (Table 4). These sequences did not show any apparent similarity to other P-450s (or other characterized proteins in the computer database examined).[4] In addition, no reactivity of any of the bands in this fraction was detected when an antibody raised to a rabbit P-450 4A protein was used (kindly provided by Drs. J. Clark and B. S. S. Masters, University of Texas, San Antonio, TX). At this time, it is not possible to identify either of these particular sequences (of the 52,000- and 58,000-Da polypeptides in that fraction) as being a P-450.

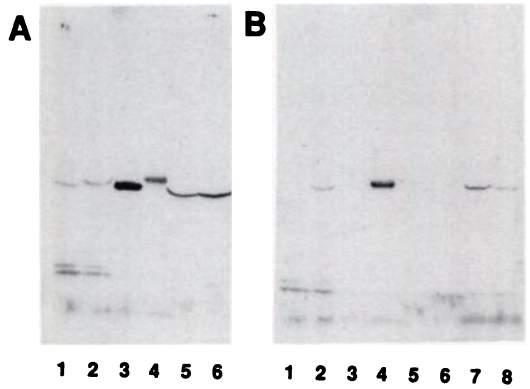

Fig. 5. SDS-polyacrylamide gel electrophoresis and immunostaining of purified and recombinant human P-450 1A1 and P-450 1A2. In this case the acrylamide gel concentration was increased (from 7.5% to 8.5%, w/v), to improve resolution. *Lanes 1* and *2*, yeast recombinant human P-450 1A1 and P-450 1A2, respectively. *Lanes 3* and *4*, purified human lung P-450 1A1 (6 μg of protein) and liver P-450 1A2 (0.5 μg of protein), respectively. *Lanes 5* and *6*, 200 μg of human lung microsomal protein (samples HLu-8 and HLu-9, respectively). *Lanes 7* and *8* (B only), 20 μg of human liver microsomal protein (samples HL 101 and HL 115, respectively). The resolved proteins were transferred to nitrocellulose sheets (14), and immunochemical staining was done with either rabbit anti-rat P-450 1A2, which recognizes both human P-450 1A1 and P-450 1A2 (A), or an autoimmune antibody (34) that recognizes human P-450 1A2 but not P-450 1A1 (B).

TABLE 3

Amino acid sequence of a peptide isolated from a purified human lung P-450

The peptide used was isolated from the HLu-10 microsomal fraction, octylamino-Sepharose fraction B, DEAE-Sephacel fractions 31–35 (Fig. 3).

Cycle	Amino acid	Recovery	Reference sequence	
			P-450 1A1 (31–48)	P-450 1A2 (35–52)
		pmol		
1	P	25	P	P
2	Q	22	Q	R
3	V	20	V	V
4	P	22	P	P
5	K	13	K	K
6	G	17	G	G
7	L	16	L	L
8	K	10	K	K
9	N	17	N	S
10	P	17	P	P
11	P	19	P	P
12	G	15	G	E
13	P	20	P	P
14	W	9	W	W
15	G	13	G	G
16	W	8	W	W
17	P	15	P	P
18	L	7	L	L

Other properties of purified human lung microsomal P-450s. P-450s were partially purified from human lung samples HLu-9 (from 75 g of lung tissue containing 360 mg of microsomal protein) and HLu-10 (from 100 g of lung tissue containing 400 mg of microsomal protein). Using the same procedure as described above, ~1.5 nmol and 2.4 nmol of P-450 were recovered from samples HLu-9 and HLu-10, respectively, after octylamino-Sepharose 4B column chromatography of the polyethylene glycol 8000 precipitate. The $Fe^{2+} \cdot CO$ versus Fe^{2+} difference spectrum of a partially purified P-450 preparation from sample HLu-10 is shown in Fig. 6. The wavelength maximum of the P-450 spectrum was observed at ~449 nm (in the spectrum of the rabbit P-450 1A2 protein, λ_{max} was 447 nm). The specific content of P-450 in lung microsomes could be calculated to be 4.2 and 6.0 pmol/mg of applied microsomal protein for samples HLu-9 and HLu-10, respectively (combined fractions A and B). Because the recovery of total P-450 from the octylamino-Sepharose 4B chromatography step is generally ~50% in the case of rat and human liver microsomes (29), the specific content should be about 10 pmol of P-450/mg of protein in human lung microsomes. The peak fractions recovered from

[4] The databank used in the searches was Swiss-Prot 19 (University of Geneva).

TABLE 4

Amino-terminal amino acid sequences of polypeptides isolated from a human lung hemoprotein fraction (octylamino-Sepharose fraction A/DEAE cellulose)

The peptides used were isolated from the HLu-10 microsomal fraction, octylamino-Sepharose fraction A, DEAE-Sephacel fractions 45–57 (Fig. 3).

Cycle	*M*, 52,000 band		*M*, 58,000 band	
	Amino acid	Recovery	Amino acid	Recovery
		pmol		*pmol*
1	T	4.0	A	6.7
2	D	3.3	E	3.5
3	L	3.7	L	5.3
4	D	1.7	R	3.3
5	I	1.5	A	4.5
6	E	1.8	P	4.2
7	A	2.8	P	4.0
8	P	1.5	D	3.1
9	F	1.0	K	3.5
10			I	3.0
11			A	2.7
12			I	2.1
13			I	2.1
14			G	2.8
15			A	2.5
16			G	2.2
17			I	2.0
18			G	1.8
19			G	1.9
20			T	2.0
21			S	1.3
22			A	1.4
23			A	1.9
24			Y	1.0
25			Y	1.6
26			L	1.2
27			R	1.0
28			Q	1.3
29			K	1.2
30			F	0.9
31			G	0.8
32			K	0.6
33			D	0.8
34			V	0.7
35			K	0.6
36			Q	0.7

the octylamino-Sepharose 4B chromatography step contained ~0.2 nmol of P-450/mg of protein, so the fold purification for this step was at least 40-fold.

The catalytic activities of partially purified P-450 preparations from human lung samples HLu-9 and HLu-10 were determined with respect to the activation of (+)- and (−)-B(*a*)P-7,8-diol and were compared with the activities of the respective lung microsomes (Table 5). The partially purified P-450 enzymes catalyzed about 50-fold higher rates of activation for both substrates than did the microsomal preparations (on the basis of protein content). However, further purification, by DEAE-Sephacel column chromatography, repeatedly caused an unexplained loss of the enzymatic activities, probably reflecting the instability of human lung P-450 1A1. The purified preparation was no longer active in the oxidization of B(*a*)P-7,8-diol, to products that were genotoxic in the *umu* assay, or in the 3-hydroxylation of B(*a*)P. In addition, the characteristic P-450 difference spectrum was lost and the preparations showed only spectra attributable to denatured cytochrome P-420. We conclude that P-450 1A1 was not lost on the column, however,

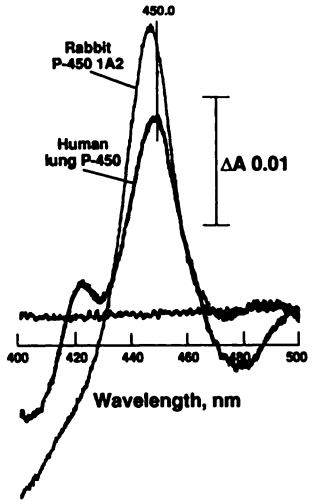

Fig. 6. Carbon monoxide difference spectra of partially purified human lung P-450 and rabbit P-450 1A2. The human lung preparation was isolated from sample HLu-10 by polyethylene glycol fractionation and octylamino-Sepharose 4B chromatography (fraction B, Fig. 5).

TABLE 5

Activation of (+)- and (−)-B(*a*)P-7,8-diols by human lung microsomes and reconstituted enzyme systems containing partially purified human lung P-450 enzymes

Means of duplicate experiments are presented.

	Rate of activation of B(*a*)P-7,8-diol			
	Sample HLu-9		Sample HLu-10	
	Microsomes	Purified P-450[a]	Microsomes	Purified P-450[a]
	umu units/min/mg of protein			
(+)-B(*a*)P-7,8-diol	12.3	71.7	3.2	88.2
(−)-B(*a*)P-7,8-diol	5.4	25.9	2.7	19.8

[a] Fraction B from octylamino-Sepharose chromatography step (followed by hydroxylapatite adsorption, washing, elution, and dialysis to remove detergent).

because of the immunochemical reaction (Figs. 4 and 5) and the isolation of a P-450 1A1 peptide (Table 3).

Characterization of P-450 enzymes in human lung microsomal preparations. SDS-polyacrylamide gel electrophoresis/immunoblotting of nine different human lung samples was conducted with rabbit anti-rat P-450 1A2 (Fig. 7). The data clearly show that all of the human lung microsomal preparations contained a protein, of the same molecular weight, that reacted with the antibodies; the molecular weight of this protein was the same as that of the purified human lung P-450 1A1.

Rates of activation of B(*a*)P-7,8-diol were measured in the presence of specific antibodies raised to purified P-450 enzymes or chemical modifiers of P-450 reactions (Fig. 8). Anti-rat P-450 1A2 inhibited the activity towards (+)-B(*a*)P-7,8-diol by ~50% in human lung microsomes (sample HLu-9) (Fig. 8A). Anti-rat P-450 1A1 was less effective in inhibiting the catalytic activity, and anti-human P-450 3A4 was without effect. A human antiserum that specifically recognizes human P-450 1A2 (34) did not inhibit the microsomal activities towards (+)- or (−)-B(*a*)P-7,8-diol in human lung sample HLu-9 (Fig. 8B). The activation of (+)-B(*a*)P-7,8-diol was completely inhibited by 7,8-benzoflavone in lung microsomes prepared from sample HLu-9 or HLu-10 (Fig. 8C). However, this chemical produced stimulation of activities of lung microsomes from sample HLu-8, a sample with a lower level of basal activity.

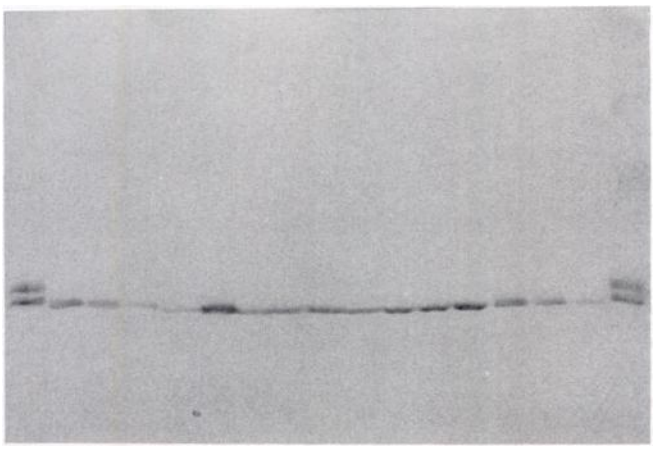

Fig. 7. SDS-polyacrylamide gel electrophoresis and immunostaining of nine different human lung microsomal samples. *Lanes 1–9*, 0.50 mg of lung microsomal protein from human samples HLu-15, -14, -13, -12, -11, -10, -9, -8, and -2, respectively. *Lane BNF*, 8 μg of liver microsomal protein isolated from BNF-treated rats. *Lanes Human lung P-450 1A1*, 4, 8, and 12 μg of a highly purified lung P-450 1A1 isolated from sample HLu-10.

Discussion

Until recently, only very weak P-450 spectra had been obtained in human lung microsomes (43). Wheeler and Guenthner (21) used a discontinuous sucrose gradient sedimentation method to remove hemoglobin and smoking-associated black debris from human lung microsomes. However, the specific content of P-450 in their samples was reported to be ~1 pmol/mg of protein (presumably these samples were obtained from smokers, because of the mention of black material). We were able to obtain typical $Fe^{2+}\cdot CO$ versus Fe^{2+} P-450 difference spectra after partial purification from human lung samples. In the two samples examined, the specific content of P-450 was estimated to be ~10 pmol/mg of protein, in line with the estimates of Prough *et al.* (43). This is ~1–2% of the specific content of P-450 in human liver.

A number of lines of evidence have indicated the presence of P-450 1A1 in human lung (22, 44). However, distinction between P-450 1A1 and P-450 1A2 is not trivial, because of the similarity in their apparent molecular weights and primary sequences (7, 42). The mRNAs are readily distinguished by size (42), and P-450 1A1 (but not P-450 1A2) mRNA has been reported to be present in human lung and induced by cigarette smoking (22). Some indirect evidence suggests that the P-450 1A1 protein is present in human lung (44), although one of the key assumptions in that work was that 7-ethoxyresorufin *O*-deethylation is catalyzed only by P-450 1A1 and not by P-450 1A2. This assumption is probably valid now, in light of all of the evidence available; however, in human liver P-450 1A2 is probably the major 7-ethoxyresorufin *O*-deethylase (34).

In this work, we report what is apparently the first P-450 to be purified from human lung microsomes. The purified protein reacted with polyclonal anti-rat P-450 1A2 but not with anti-rat P-450 1A1 (Figs. 4 and 5) or a human autoimmune antibody that recognizes only human P-450 1A2 and not human P-450 1A1. Comparison of the electrophoretic migration patterns of the recombinant and the human proteins also provided evidence that the purified protein is P-450 1A1 (Fig. 5). Further, a peptide isolated from the purified protein was clearly shown to correspond to P-450 1A1 and not to P-450 1A2 (Table 3). The partially purified preparation catalyzed the activation of both (+)- and (−)-B(*a*)P-7,8-diol, but upon further purification neither the $Fe^{2+}\cdot CO$ versus Fe^{2+} spectrum nor catalytic activity was recovered. This instability may be an inherent property of human P-450 1A1.

The levels of catalytic activities measured in human lung microsomes were considerably lower than those in rats (Table 1). However, immunoblotting studies suggest that all of the

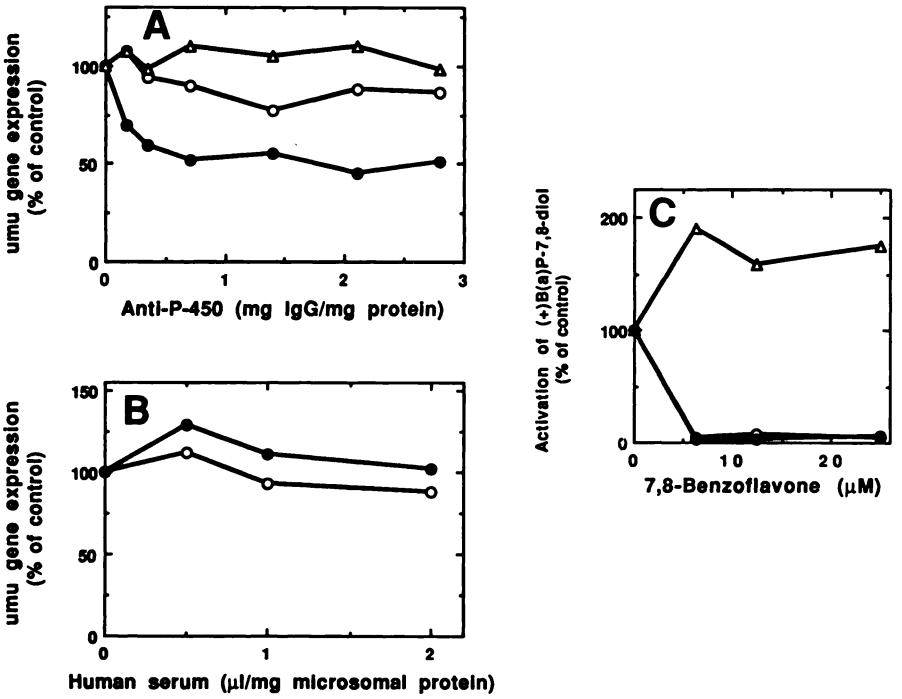

Fig. 8. Inhibition of catalytic activities of human lung microsomes. A, Effects of antibodies on the activation of (+)-B(*a*)P-7,8-diol in human lung microsomes. Human lung microsomes (sample HLu-9) were mixed with the indicated amount of rabbit anti-human P-450 3A4 (△), anti-rat P-450 1A1 (○), or anti-rat P-450 1A2 (●), and activity was measured. The uninhibited rate was 10 *umu* units/min/mg of microsomal protein. B, Effect of anti-human P-450 1A2 on the activation of (+)- or (−)-B(*a*)P-7,8-diol. The indicated amount of the human autoimmune antibodies was added to human lung microsomes (sample HLu-9). The basal activity was 9.5 and 6.5 *umu* units/min/mg of microsomal protein for (+)-B(*a*)P-7,8-diol (●) and (−)-B(*a*)P-7,8-diol (○), respectively. C, Effect of 7,8-benzoflavone on the activation of (+)-B(*a*)P-7,8-diol in different human lung microsomal preparations. The basal rates of activation were 3.6, 11.7, and 5.6 *umu* units/min/mg of microsomal protein for samples HLu-8 (△), HLu-9 (○), and HLu-10 (●), respectively.

human lung samples contained P-450 1A1. Whether this finding is influenced by the fact that these surgical samples were collected from individuals who had some kind of lung disease is not clear; some individuals appear to have only small amounts of lung P-450 1A1 mRNA (22). Polyclonal anti-rat P-450 1A2 and anti-P-450 1A1 were able to inhibit the activation of (+)-B(*a*)P-7,8-diol in a sample of human lung microsomes (Fig. 8A), and this inhibition is attributed to the reaction of these antibodies with human P-450 1A1, not P-450 1A2. The autoimmune serum, which is very inhibitory to human liver microsomal phenacetin *O*-deethylation activity (34) and recognizes mainly human P-450 1A2, was without effect on the activation of (+)-B(*a*)P-7,8-diol in human lung microsomes (Fig. 8B). Catalytic activities towards the substrates B(*a*)P, (+)- and (−)-B(*a*)P-7,8-diol, and 7-ethoxyresorufin correlated well with each other in the set of human lung microsomal samples used in this study (Table 1). However, these activities did not correlate particularly well with the apparent level of P-450 1A1 measured by immunoblotting analysis (Fig. 7). At this time, we do not have a completely satisfying explanation for the discrepancy (see also Ref. 44); however, the apparent instability of human lung P-450 1A1 observed in the purification work (see above) suggests that the enzyme might not be completely stable in the lung samples and might lose activity. Rat and rabbit P-450 1A1 enzymes appear to be reasonably stable in our experience (30); however, yeast recombinant human P-450 1A1 seems to be less stable than human P-450 1A2 in the work done to date.[3]

No real evidence was obtained for the expression of P-450 1A2 in human lung. As pointed out before, it is difficult to distinguish human P-450 1A1 and P-450 1A2 on the basis of their migration in SDS-polyacrylamide gels (45) or their immunoreactivity (44). The autoimmune antibody, which seems to be specific for P-450 1A2, did not recognize purified P-450 1A1 or any other polypeptides in human lungs. There was no detectable phenacetin *O*-deethylation activity in any of the human lung samples examined (Table 1). Further, neither of the two human lung microsomal samples investigated was able to catalyze the activation of IQ, MeIQ, or Trp P-2 to genotoxic products (Table 2). Thus, human lung microsomes seem to differ from rat lung microsomes, which appear to have some P-450 1A2 (apparently not inducible), as judged by direct immunochemical assays (13) and the activity measurements presented here (Table 1). It is also of interest to note that the related compound Trp P-1, which differs from Trp P-2 only by the presence of an additional methyl group, appears to be *N*-hydroxylated primarily by P-450 1A2 in rat liver (46) but is activated primarily by other enzymes in human liver (28). Thus, caution should be exercised in applying all conclusions from one species to another.

Another matter of interest is the effect of 7,8-benzoflavone on the activation of (+)-B(*a*)P-7,8-diol (Fig. 8C). The compound could inhibit or stimulate, depending upon the particular lung sample used. We had previously noted the ability of 7,8-benzoflavone to stimulate this activity in human lung microsomes (24). 7,8-Benzoflavone is a potent inhibitor of P-450 1A family enzymes, and, in the samples in which inhibition is seen, P-450 1A1 is considered to be the major catalyst of the oxidation reaction. In the samples in which stimulation is observed, we feel that other enzymes, possibly P-450 3A enzymes (24), make a large contribution to the activity. There was some relationship between the absolute level of basal activity towards (+)-B(*a*)P-7,8-diol benzoflavone, but it was not a strong correlation (Fig. 8C; Table 1). This problem might also be a reflection of deterioration of activity of P-450 1A1 in the human lung samples.

In conclusion, these studies indicate that P-450 1A1 has a significant role in the metabolism of several carcinogens in human lung. P-450 1A1 is inducible (22); there was no evidence for the presence of P-450 1A2 in human lung and it is probably not inducible. There are other P-450 enzymes present in human lung, and their roles in the activation of other carcinogens are still speculative. One of the enzymes was partially purified (Fig. 3) and seems to be present at fairly high levels, although we cannot at this time state how many P-450 proteins are present in the preparation or whether either of the two peptides for which sequence information is presented is actually a P-450 protein. Finally, it should be emphasized that, although characterization of the enzymes in the lungs of humans and experimental animals is important, knowledge concerning these enzymes must be put in the context of metabolism of carcinogens in the liver and other tissues, because evidence exists that many of the carcinogens may well be able to migrate to target sites after activation to proximate and ultimate forms (47).

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